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Keywords: Natural Products; Anticancer Properties; Cancer; medicinal plants; Chemical profile



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## Article

# Chemical Composition, Antioxidant, and Cytotoxic Effects of *Senna rugosa* Leaf and Root Extracts on Human Leukemia Cell Lines

Cintia M. dos Santos <sup>1</sup>, Debora da Silva Baldivia <sup>1</sup>, David T. H. de Castro <sup>1</sup>, José T. de G. Carvalho <sup>1</sup>, Alex S. Oliveira <sup>1</sup>, Paola dos Santos da Rocha <sup>1</sup>, Jaqueline Ferreira Campos <sup>1</sup>, Sikiru Olaitan Balogun <sup>1,2</sup>, Caio F. R. de Oliveira <sup>1</sup>, Denise Brentan Silva <sup>3</sup>, Carlos A. Carollo <sup>3</sup>, Kely de P. Souza <sup>1</sup> and Edson L. dos Santos <sup>1,2,\*</sup>

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**Abstract:** *Senna rugosa* is a species found in the Cerrado and used in folk medicine as a vermifuge and in the treatment of poisonous snakebites accidents. In this work, we identified the main secondary metabolites present in ethanolic extracts of the leaves (ELSR) and roots (ERSR) of *S. rugosa* and evaluated the potential cytoprotective effect against cellular macromolecular damage, as well as the cytotoxic properties of the extracts on the K562 and Jurkat leukemic cell lines. The identification of metabolites was done by liquid chromatography coupled with mass spectrometry. The antioxidant activities were investigated by direct ABTS<sup>•+</sup> and DPPH<sup>•</sup> radical scavenging methods, protection against oxidative damage in proteins and DNA. The cytotoxic properties were investigated against healthy cells, isolated from human peripheral blood (PBMC) and leukemic cell lines. The leaf extract contains catechin, rutin, epigallocatechin derivatives, kaempferol glycosides, luteolin, and dimeric and trimeric procyanidins, while the root extract profile shows obtusichromoneside derivatives, 2-methoxystypandrone, stilbene derivatives, naphthopyranones, and flavanone derivatives. The extracts showed antioxidant activity, with IC<sub>50</sub> of 4.86 ± 0.51 µg/mL and 8.33 ± 0.90 µg/mL in the ABTS assay for ELSR and ERSR, respectively. And in the DPPH<sup>•</sup> assay, the IC<sub>50</sub> was 19.98 ± 1.96 µg/mL for ELSR and 13.37 ± 1.05 µg/mL for ERSR. The extracts protected macromolecules against oxidative damage at concentrations of 5 µg/mL. The cytotoxicity test against leukemic strains was observed after 24 and 48 hours of treatment. After 48 h, results against the K562 cell line demonstrate IC<sub>50</sub> of 242.54 ± 2.38 µg/mL and 223.00 ± 2.34 µg/mL for ELSR and ERSR, respectively. While against the Jurkat cell line, these extracts showed IC<sub>50</sub> of 171.45 ± 2.25 µg/mL and 189.30 ± 2.27 µg/mL, respectively. Results on PBMC viability, demonstrate that the extracts showed selectivity for the leukemic cell lines. Together, our results reveal that the leaves and roots of *S. rugosa* have completely distinct and complex chemical compositions. This study expands the repertoire of identified chemical compounds and underscores their significant pharmacological potential in conditions related to oxidative stress and leukemia.

**Keywords:** natural products; anticancer properties; cancer; medicinal plants; chemical profile

## 1. Introduction

Oxidative stress is characterized by an imbalance between reactive species and the endogenous antioxidant system [1,2]. The oxidative damage on macromolecules - proteins and nucleic acids, accentuates cellular aging and reduces the efficiency of DNA repair mechanisms, increasing the accumulation of mutations [3,4], associated with the development of pathologies such as cancer, among others [5]. The deleterious effects of oxidative stress can be controlled by antioxidants, conferring protection against reactive species of endogenous or exogenous origin [6,7]. The beneficial effects of antioxidants on physiology are widely known [8]. Furthermore, antioxidants can promote pro-oxidant effects in cancer cells by activating different cell death mechanisms [9,10].

Leukemia is a hematologic neoplasm affecting the hematopoietic system's cells [11]. Four main subtypes of leukemias are known and diagnosis varies according to the maturity and type of cell lineage [12]. In many cases of leukemias, translocations between chromosomes 9 and 22 are observed, leading to the fusion of the BCR and ABL1 genes. This fusion results in increased tyrosine-kinase activity [13] and the activation of several signaling pathways, such as Jak/Stat and Ras/Raf/Map-kinase, resulting in uncontrolled events during the cell cycle [14]. Lack of control over cell cycle progression can also occur due to silencing of suppressor (p53 and PTEN) and pro-apoptotic genes (BAX) [15].

Despite advances in the treatment of leukemia, about 20% of patients have a relapse rate of the disease or adverse reactions to treatment, highlighting the importance of developing new therapies [11,16]. Consequently, a high mortality rate is recorded. In 2020, more than 19 million cases occurred worldwide, with a mortality rate of 70% [17]. In this regard, the scientific community has intensified the search for new therapeutic agents with anticancer activity of natural origin. Recent studies have demonstrated the pharmacological potential of plants from the Brazilian Cerrado, a biome with the potential for discovering new phytochemicals [18].

Recent pharmacological studies have shown that the crude extracts or derivatives of the *Senna* genus possess, among others, antidiabetic, antioxidant, anti-inflammatory, antitumor, and anticancer activities [19–21]. Several studies on medicinal plants from Cerrado have presented crude plant extracts with anticancer properties [22–25]. The extracts of these species presented a varied composition of secondary metabolites, among them gallic acid, catechin, epigallocatechin, epicatechin, kaempferol heteroside, rutin, ellagic acid, anthraquinone, piceatanol, dimeric and trimeric proanthocyanidins. Several studies have demonstrated the effect of these compounds on their cytotoxic effects on cancer cells [26]. *Senna rugosa* (Fabaceae) is a plant species popularly known as *amendoirana*, *alcacuz bravo*, *bico-de-corvo*, *boi gordo* and *paratudo*, where its seeds are used to treat parasitic worms' infestations [27,28] and its roots are used in the treatment of poisonous snake bites [29]. Recently, Cunha et al., [30] evaluated the chemical composition of the ethanolic extract of *S. rugosa* leaves and fractions obtained from the extract and identified the presence of mainly phenolic compounds, which include flavonols, anthraquinones and anthrones derivatives (particularly, rutin, emodin, aloe-emodin and barbaloin/isobarbaloin). In that study, antimicrobial, antifungal, and moderate antitumor activities against breast cancer cell lines were reported. There are reports that several species of the *Senna* genus possess anticancer activities against various tumor cell lines [21,31].

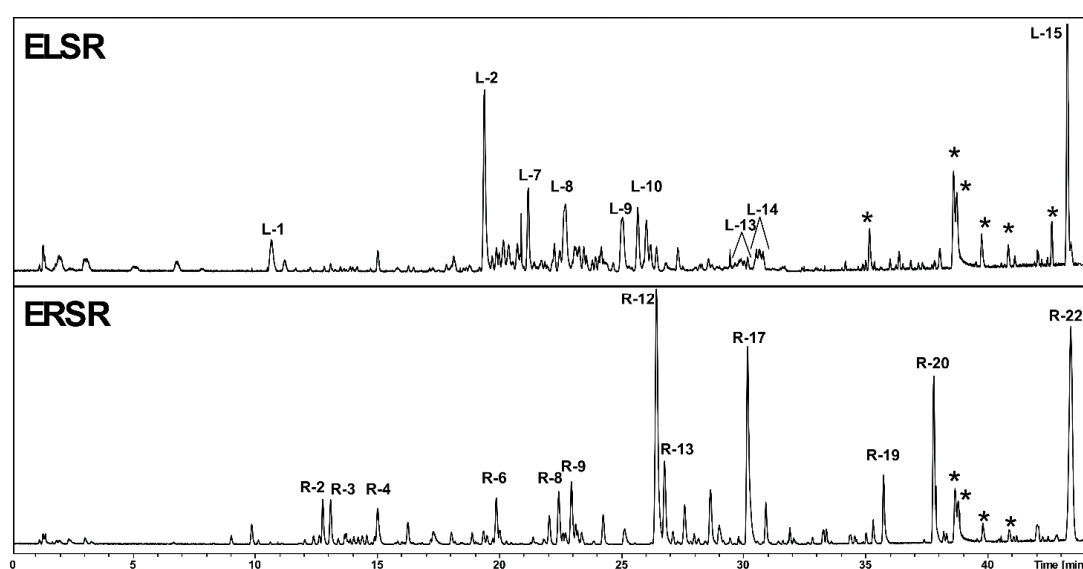
As the species has been used by the population in the treatment of pathologies, we proposed to determine the chemical constitution of the metabolites present in the leaves and roots of *S. rugosa*. In addition, we investigated the antioxidant properties and cytotoxic activities of the ethanolic extracts of *S. rugosa* leaves (ELSR) and roots (ERSR) against chronic (K562) and acute (Jurkat) human leukemia cell lines. We used human peripheral blood cells (PBMC) in this study to determine the selectivity of the extracts on leukemic cells compared to healthy human cells.

## 2. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

### 2.1. Chemical Composition of ELSR

The detailed phytochemical analysis of *S. rugosa*, conducted separately on its leaf extracts (Table 1 - ELSR) and root extracts (Table 1 - ERSR), revealed distinct chemical profiles between these two plant organs. In the leaf extracts, we identified a variety of flavan-3-ol dimers and trimers, epiafzelechin derivatives, and a range of flavonoids, including rutin, luteolin, and kaempferol 3-O-rutinoside, indicating a marked predominance of phenolic and flavonoids derivatives. In contrast, the root extracts presented a widely varied secondary metabolic, ranging from C-glycosylated chromones, notably of the obtusichromoneside class, to glycosylated naphthopyranones with skeletons compatible with rubrofusarin derivatives, stilbenoids, and possible glycosylated and sulfated flavonoids. The chromatographic profile is shown in Figure 1, while the retention times, molecular formula, and M+H are in Table 1.



**Figure 1.** Chemical profile of ethanolic extracts of the leaves (ELSR) and roots (ERSR) of *S. rugosa* represented by chromatogram in column C-18. \* Column contaminants.

**Table 1.** Chemical profile of ethanolic extracts of the leaves (ELSR) and roots (ERSR) of *S. rugosa* analyzed using UFLC-MS.

ELSR							
Peak	Time(min)	UV	Formula	[M+H] <sup>+</sup>	MS/MS	Classes	Compound
L-1	10.6	280	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	291.0870	-	Flavan-3-ol	Catechin
L-2	19.4	256/353	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	611.1599	465 (C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> ), 449 (C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> ), 303 (C <sub>15</sub> H <sub>11</sub> O <sub>7</sub> )	Flavonol	Rutin
L-3	19.8	313	C <sub>15</sub> H <sub>14</sub> O <sub>5</sub>	275.0914	225 (C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> ), 213 (C <sub>13</sub> H <sub>9</sub> O <sub>3</sub> ), 197 (C <sub>13</sub> H <sub>9</sub> O <sub>2</sub> ), 185 (C <sub>12</sub> H <sub>9</sub> O <sub>2</sub> ), 157 (C <sub>11</sub> H <sub>9</sub> O)	Stilbene	3,3',5,5'-Tetrahydroxy-4-methoxystilbene
L-4	20.1	280	C <sub>30</sub> H <sub>26</sub> O <sub>10</sub>	547.1580	393 (C <sub>22</sub> H <sub>17</sub> O <sub>7</sub> ), 285 (C <sub>16</sub> H <sub>13</sub> O <sub>5</sub> ), 271 (C <sub>15</sub> H <sub>11</sub> O <sub>5</sub> ), 259 (C <sub>14</sub> H <sub>11</sub> O <sub>5</sub> ), 241 (C <sub>14</sub> H <sub>9</sub> O <sub>4</sub> ), 147 (C <sub>9</sub> H <sub>7</sub> O <sub>2</sub> )	Proanthocyanidin	Epiafzelechin-epiafzelechin derivative
L-5	20.3	280	C <sub>30</sub> H <sub>26</sub> O <sub>10</sub>	547.1582	393 (C <sub>22</sub> H <sub>17</sub> O <sub>7</sub> ), 271 (C <sub>15</sub> H <sub>11</sub> O <sub>5</sub> ), 241 (C <sub>14</sub> H <sub>9</sub> O <sub>4</sub> )	Proanthocyanidin	Epiafzelechin-epiafzelechin derivative
L-6	20.7	280	C <sub>30</sub> H <sub>26</sub> O <sub>10</sub>	547.1589	409 (C <sub>22</sub> H <sub>17</sub> O <sub>8</sub> ), 269 (C <sub>16</sub> H <sub>13</sub> O <sub>4</sub> ), 243 (C <sub>14</sub> H <sub>11</sub> O <sub>4</sub> ), 163 (C <sub>9</sub> H <sub>7</sub> O <sub>3</sub> )	Proanthocyanidin	Epiafzelechin-epiafzelechin derivative
L-7	21.1	265/347	C <sub>27</sub> H <sub>30</sub> O <sub>15</sub>	595.1651	449 (C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> ), 287 (C <sub>15</sub> H <sub>11</sub> O <sub>6</sub> )	Flavonol	Kaempferol 3-O-rutinoside
L-8	22.6	280	C <sub>30</sub> H <sub>26</sub> O <sub>9</sub>	531.1644	393 (C <sub>22</sub> H <sub>17</sub> O <sub>7</sub> ), 269 (C <sub>16</sub> H <sub>13</sub> O <sub>4</sub> )	Proanthocyanidin	Trihydroxyflavan-epiafzelechin derivative
L-9	24.9	280	C <sub>30</sub> H <sub>26</sub> O <sub>9</sub>	531.1648	393 (C <sub>22</sub> H <sub>17</sub> O <sub>7</sub> ), 269 (C <sub>16</sub> H <sub>13</sub> O <sub>4</sub> )	Proanthocyanidin	Trihydroxyflavan-epiafzelechin derivative
L-10	25.6	280	C <sub>30</sub> H <sub>26</sub> O <sub>9</sub>	531.1648	393 (C <sub>22</sub> H <sub>17</sub> O <sub>7</sub> ), 269 (C <sub>16</sub> H <sub>13</sub> O <sub>4</sub> ), 243 (C <sub>14</sub> H <sub>11</sub> O <sub>4</sub> ), 207 (C <sub>11</sub> H <sub>11</sub> O <sub>4</sub> )	Proanthocyanidin	Trihydroxyflavan-epiafzelechin derivative
L-11	25.9	277/345	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	287.0546	241 (C <sub>14</sub> H <sub>9</sub> O <sub>4</sub> ), 153 (C <sub>7</sub> H <sub>5</sub> O <sub>4</sub> )	Flavone	Luteolin
L-12	26.3	280	C <sub>30</sub> H <sub>26</sub> O <sub>9</sub>	531.1651	-	Proanthocyanidin	Trihydroxyflavan-epiafzelechin derivative
L-13	27.2	279/350	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	317.0662	-	-	3-metoxyluteolin
L-14	29.2-30.1	280	C <sub>45</sub> H <sub>38</sub> O <sub>13</sub>	787.2360	-	Proanthocyanidin	Trimeric procyanidins
L-15	30.2-30.9	280	C <sub>45</sub> H <sub>38</sub> O <sub>12</sub>	771.2412	-	Proanthocyanidin	Trimeric procyanidins
L-16	43.2	406	C <sub>34</sub> H <sub>40</sub> O <sub>9</sub>	593.2750	533 (C <sub>32</sub> H <sub>37</sub> O <sub>7</sub> ), 461 (C <sub>29</sub> H <sub>33</sub> O <sub>5</sub> )	Tetrahydroanthracene	Dimeric tetrahydroanthracene derivative
ERSR							



Peak	Time (min)	UV	Formula	[M+H] <sup>+</sup>	MS/MS	Classes	Compound
R-1	9.8	253/298	C <sub>24</sub> H <sub>32</sub> O <sub>15</sub>	561.1791	489 (C <sub>24</sub> H <sub>25</sub> O <sub>11</sub> ), 423 (C <sub>20</sub> H <sub>23</sub> O <sub>10</sub> ), 345 (C <sub>18</sub> H <sub>17</sub> O <sub>7</sub> ), 291 (C <sub>15</sub> H <sub>15</sub> O <sub>6</sub> )	-	unknown
R-2	12.7	254/299	C <sub>20</sub> H <sub>26</sub> O <sub>11</sub>	443.1542	323 (C <sub>16</sub> H <sub>19</sub> O <sub>7</sub> ), 293 (C <sub>15</sub> H <sub>17</sub> O <sub>6</sub> ), 235 (C <sub>12</sub> H <sub>11</sub> O <sub>5</sub> ), 205 (C <sub>11</sub> H <sub>9</sub> O <sub>4</sub> )	Chromone	Obtusichromoneside derivative
R-3	13	252/295	C <sub>20</sub> H <sub>26</sub> O <sub>11</sub>	443.1548	371 (C <sub>20</sub> H <sub>19</sub> O <sub>7</sub> ), 259 (C <sub>14</sub> H <sub>11</sub> O <sub>5</sub> ), 235 (C <sub>12</sub> H <sub>11</sub> O <sub>5</sub> ), 205 (C <sub>11</sub> H <sub>9</sub> O <sub>4</sub> )	Chromone	Obtusichromoneside derivative
R-4	14.9	320	C <sub>14</sub> H <sub>12</sub> O <sub>5</sub>	261.0757	197 (C <sub>13</sub> H <sub>9</sub> O <sub>2</sub> ), 169 (C <sub>12</sub> H <sub>9</sub> O)	Naphthoquinone	2-Methoxystypandrone
R-5	16.2	313	C <sub>15</sub> H <sub>14</sub> O <sub>5</sub>	275.091	213 (C <sub>13</sub> H <sub>9</sub> O <sub>3</sub> ), 197 (C <sub>13</sub> H <sub>9</sub> O <sub>2</sub> ), 169 (C <sub>12</sub> H <sub>9</sub> O)	Stilbene	Tetrahydroxy-methoxy stilbene derivative
R-6	19.8	313	C <sub>15</sub> H <sub>14</sub> O <sub>5</sub>	275.0914	225 (C <sub>14</sub> H <sub>9</sub> O <sub>3</sub> ), 213 (C <sub>13</sub> H <sub>9</sub> O <sub>3</sub> ), 197 (C <sub>13</sub> H <sub>9</sub> O <sub>2</sub> ), 185 (C <sub>12</sub> H <sub>9</sub> O <sub>2</sub> ), 157 (C <sub>11</sub> H <sub>9</sub> O)	Stilbene	3,3',5,5'-Tetrahydroxy-4- methoxystilbene
R-7	22	280/334	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	303.0867	271 (C <sub>15</sub> H <sub>11</sub> O <sub>5</sub> ), 243 (C <sub>14</sub> H <sub>11</sub> O <sub>4</sub> ), 203 (C <sub>11</sub> H <sub>7</sub> O <sub>4</sub> )	Chromone	chromone deriative
R-8	22.3	278/326/400	C <sub>25</sub> H <sub>28</sub> O <sub>14</sub>	553.1554	259 (C <sub>14</sub> H <sub>11</sub> O <sub>5</sub> )	Naphthopyrone	Putative norrubrofusarin gentiobioside
R-9	22.9	279/312/366	C <sub>26</sub> H <sub>30</sub> O <sub>14</sub>	567.1699	273 (C <sub>15</sub> H <sub>13</sub> O <sub>5</sub> )	Flavanone	Putative hexahydroxy Flavanonol pentosyl-hexosyl
R-10	24.2	277/324/399	C <sub>27</sub> H <sub>32</sub> O <sub>15</sub>	597.18	273 (C <sub>15</sub> H <sub>13</sub> O <sub>5</sub> )	Naphthopyrone	Rubrofusarin gentiobioside
R-11	25.1	276/310/369	C <sub>15</sub> H <sub>12</sub> O <sub>8</sub> S	353.0314	273 (C <sub>15</sub> H <sub>13</sub> O <sub>5</sub> ), 230 (C <sub>13</sub> H <sub>10</sub> O <sub>4</sub> )	Flavanone	Putative hexahydroxy Flavanonol sulfate
R-12	26.4	277/324/395	C <sub>26</sub> H <sub>30</sub> O <sub>14</sub>	567.1709	273 (C <sub>15</sub> H <sub>13</sub> O <sub>5</sub> )	Naphthopyrone	Cassiaside B
R-13	26.7	285/320/379	C <sub>27</sub> H <sub>32</sub> O <sub>14</sub>	581.1864	449 (C <sub>22</sub> H <sub>25</sub> O <sub>10</sub> ), 287 (C <sub>16</sub> H <sub>15</sub> O <sub>5</sub> )	Flavanone	Putative hexahydroxy-methoxy Flavanonol pentosyl-hexosyl
R-14	27.5	277/324/401	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	435.1286	273 (C <sub>15</sub> H <sub>13</sub> O <sub>5</sub> )	Naphthopyrone	Rubrofusarin-O-glucopyranoside
R-15	28.6	279/330/413	C <sub>28</sub> H <sub>34</sub> O <sub>15</sub>	611.1963	449 (C <sub>22</sub> H <sub>25</sub> O <sub>10</sub> ), 287 (C <sub>16</sub> H <sub>15</sub> O <sub>5</sub> )	-	unknown
R-16	28.9	285/321/377	C <sub>16</sub> H <sub>14</sub> O <sub>8</sub> S	367.0472	287 (C <sub>16</sub> H <sub>15</sub> O <sub>5</sub> )	Flavanone	Putative hexahydroxy-methoxy Flavanonol sulfate
R-17	30.1	258/278/331/411	C <sub>27</sub> H <sub>32</sub> O <sub>14</sub>	581.1861	449 (C <sub>22</sub> H <sub>25</sub> O <sub>10</sub> ), 419 (C <sub>21</sub> H <sub>23</sub> O <sub>9</sub> ), 287 (C <sub>16</sub> H <sub>15</sub> O <sub>5</sub> )	-	unknown
R-18	30.8	279/330/411	C <sub>22</sub> H <sub>24</sub> O <sub>10</sub>	449.1433	287 (C <sub>16</sub> H <sub>15</sub> O <sub>5</sub> )	-	unknown
R-19	35.7	277/325/402	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	273.0758	230 (C <sub>13</sub> H <sub>10</sub> O <sub>4</sub> )	Naphthopyrone	Rubrofusarin

R-20	37.7	281/337/425	C <sub>16</sub> H <sub>15</sub> O <sub>5</sub>	287.0917	272 (C <sub>15</sub> H <sub>12</sub> O <sub>5</sub> ), 254 (C <sub>15</sub> H <sub>10</sub> O <sub>4</sub> ), 244 (C <sub>14</sub> H <sub>12</sub> O <sub>4</sub> ), 226 (C <sub>15</sub> H <sub>10</sub> O <sub>3</sub> ), 198 (C <sub>13</sub> H <sub>10</sub> O <sub>2</sub> )	-	unknown
R-21	43.4	410	C <sub>36</sub> H <sub>44</sub> O <sub>9</sub>	621.308	561 (C <sub>34</sub> H <sub>41</sub> O <sub>7</sub> )	-	unknown
R-22	44.5	410	C <sub>36</sub> H <sub>44</sub> O <sub>9</sub>	621.3077	561 (C <sub>34</sub> H <sub>41</sub> O <sub>7</sub> )	-	unknown

2.2. Antioxidant Activity Assays

Table 2 presents the IC<sub>50</sub> values for the ABTS<sup>•+</sup> and DPPH<sup>•</sup> radicals, as well as the concentrations at which the ELSR and ERSR showed the maximum antioxidant activity. For the ABTS<sup>•+</sup> radical, ELSR presented IC<sub>50</sub> 1.25 times lower when compared with the reference antioxidant BHT, presenting maximum activity at the same concentrations (50 µg/mL). ERSR presented IC<sub>50</sub> 1.37 times higher than BHT, however, its maximum activity was reached at half the concentration of BHT.

In the direct DPPH<sup>•</sup> radical scavenging assay, the IC<sub>50</sub> of EFRS and ERSR were 3.5 and 5.3 times lower, respectively, than that calculated for BHT, demonstrating a higher antioxidant activity of the extracts compared to this reference compound. Both extracts showed maximum activity at concentrations 5 times lower than BHT.

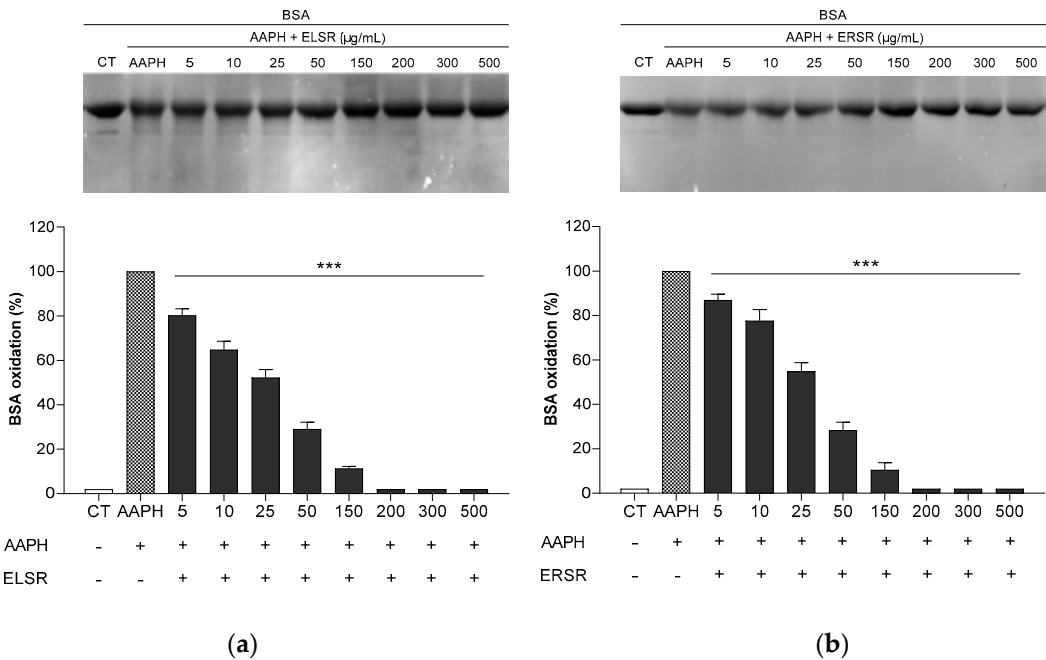
**Table 2.** IC<sub>50</sub> values and maximum activity of the ELSR, ERSR, AA, and BHT.

Samples	ABTS <sup>•+</sup>			DPPH <sup>•</sup>		
	IC <sub>50</sub> (µg/mL)	Maximum Activity (%)	(µg/mL)	IC <sub>50</sub> (µg/mL)	Maximum Activity (%)	(µg/mL)
AA	1.32 ± 0.04	99.28 ± 0.16	5	2.35 ± 0.34	94.88 ± 0.16	10
BHT	6.08 ± 0.77	98.98 ± 0.24	50	71.86 ± 2.32	93.16 ± 0.23	500
ELSR	4.86 ± 0.51	98.15 ± 0.53	50	19.98 ± 1.96	95.24 ± 0.20	100
ERSR	8.33 ± 0.90	98.40 ± 0.33	25	13.37 ± 1.05	94.98 ± 0.27	100

Values are expressed as mean ± SEM (n=3). AA = ascorbic acid; BHT = butylated hydroxytoluene.

2.3. Protection of ELSR and ERSR against Oxidative Damage in Macromolecules

ELSR and ERSR protected BSA protein against AAPH-induced oxidation at all concentrations evaluated (Figure 2). The images of the gels are representative and demonstrate the protection against oxidation after ELSR and ERSR treatments. Both extracts reduced protein oxidation, resulting from AAPH exposure when compared to the AAPH control, where BSA was incubated with AAPH alone.

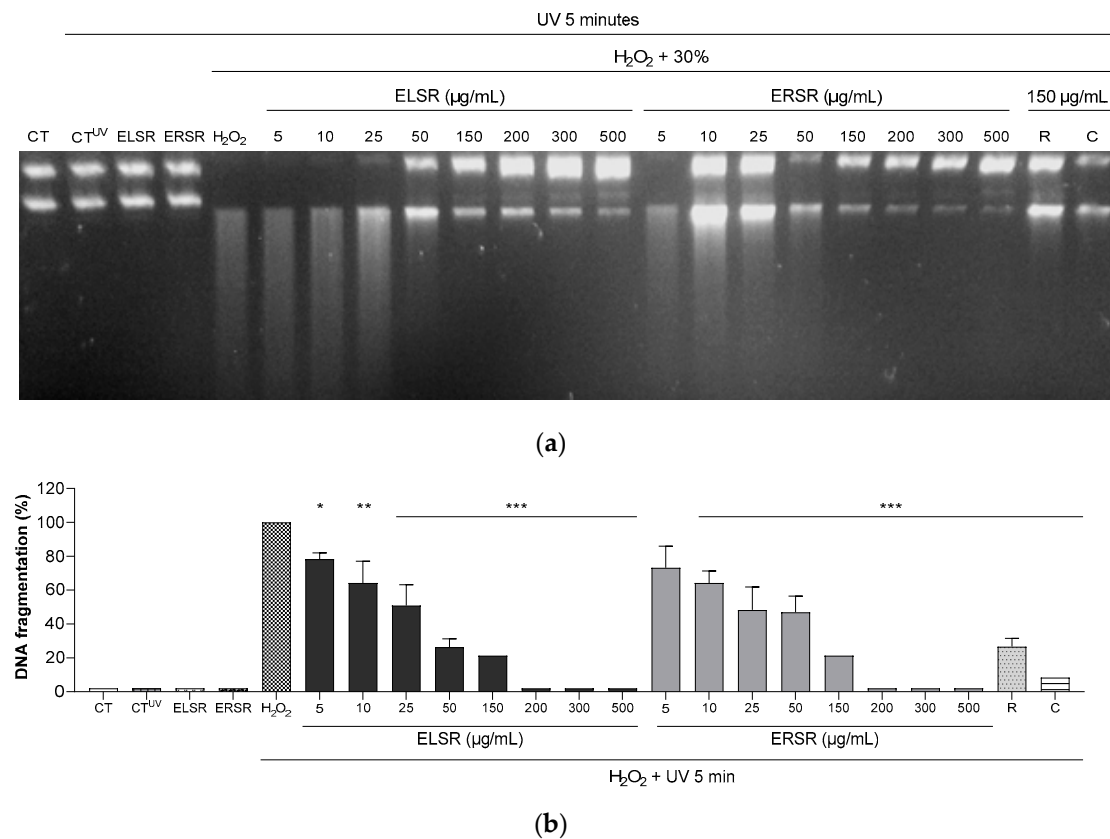


**Figure 2.** Effect of the protection promoted by the extracts against AAPH-induced oxidation of BSA. BSA was incubated with different concentrations of ELSR (a) and ERSR (b). Data present results from



four independent experiments, \*\*\* p < 0.0001 compared to control BSA incubated with AAPH alone (AAPH). CT: BSA control.

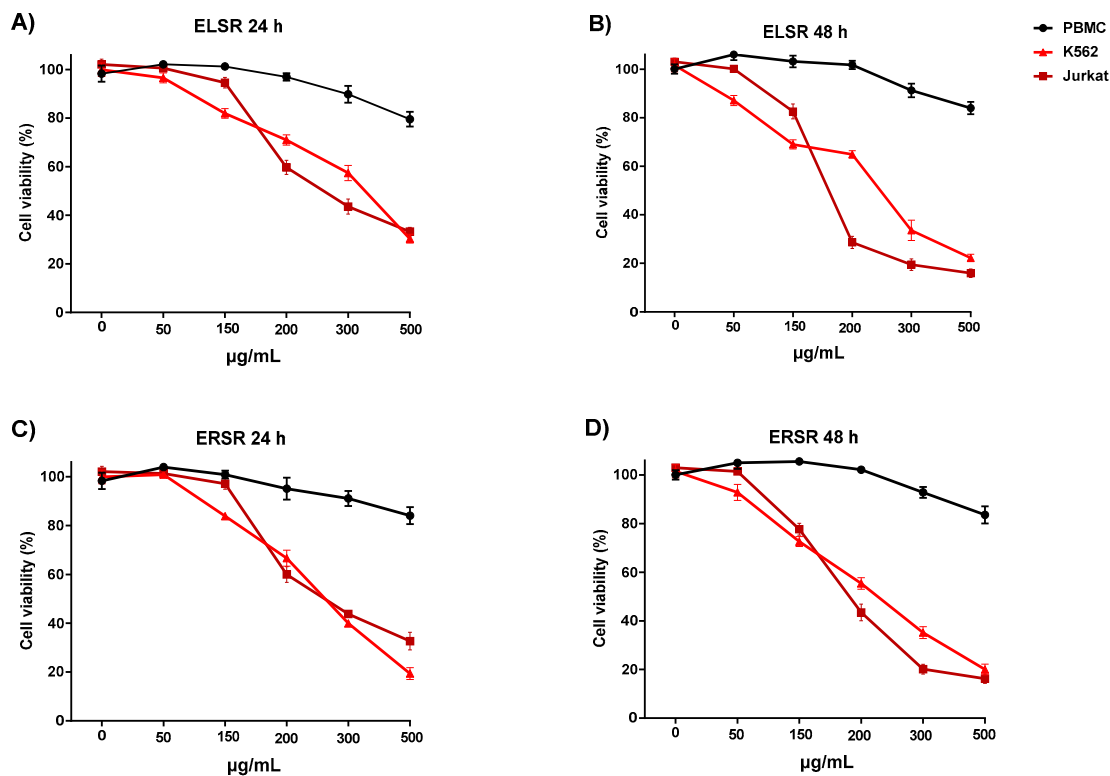
ELSR and ERSR were able to protect DNA from H<sub>2</sub>O<sub>2</sub> UV-induced fragmentation in a concentration-dependent manner (Figure 3-B). Figure 3A is a representative picture of the protection against fragmentation after ELSR and ERSR treatments. In the absence of extracts and at low concentrations (up to 25 µg/mL) fragmentation of plasmid DNA is observed. Reduction of fragmentation is visible from 50 µg/mL onwards. The reference antioxidants rutin and catechin caused a reduction in DNA fragmentation, however, rutin was more effective in the evaluated concentration.



**Figure 3.** Effect of ELSR and ERSR protection against DNA fragmentation induced by H<sub>2</sub>O<sub>2</sub> and UV radiation. Representative image of plasmid DNA bands (a). Percent fragmentation of plasmid DNA incubated with different concentrations (5-500 µg/mL) of ELSR and ERSR (b). Results expressed a percentage of DNA fragmentation. PC: Plasmid control; PCUV: Plasmid + UV control; ELSR: Plasmid + ELSR 500 µg/mL; ERSR: Plasmid + ERSR 500 µg/mL; H<sub>2</sub>O<sub>2</sub>: plasmid + H<sub>2</sub>O<sub>2</sub> 30%; R: rutin; C: catechin. Data present results from three independent experiments,\* p < 0.05; \*\* p < 0.01, and \*\*\* p < 0.001 compared to control DNA incubated with H<sub>2</sub>O<sub>2</sub> and subjected to UV.

2.4. Cytotoxicity against Leukemic Strains

PBMC cells and K562 and Jurkat leukemic cell lines were treated with different concentrations of the ELSR and ERSR for 24 and 48 h. Incubation of the ELSR and ERSR (Figure 4 A–D) with PBMC showed a minimal reduction in the cell viability of 17% on average, but only at the highest concentration of the extracts. None of the concentrations of the extracts caused a 50% reduction in the PBMC viability, suggesting that the extracts are selective to the leukemic cells. The result with the solvent control (0.1% ethanol) demonstrated that the cells' viability under these conditions was unaffected by the solvent. The results show that the extracts showed a cytotoxic effect against the leukemic cell lines in a concentration-dependent manner, with the K562 cell line being less sensitive than the Jurkat cell line, as observed through the IC<sub>50</sub> values presented in Table 3.



**Figure 4.** Cell viability of PBMC, K562, and Jurkat cell lines after 24 (A, C) and 48 h (B, D) of treatment with different concentrations of the ELSR and ERSR (50 - 500 µg/mL). Data were expressed as the means ± SEM of three independent experiments. Results were considered significant when \*\*\*\* p< 0.0001 compared to control (CT) cells incubated with culture medium only.

**Table 3.** IC<sub>50</sub> values were found for human leukemia cell lines K562 and Jurkat after treatment with ELSR and ERSR.

Cell line	ELSR		ERSR	
	IC <sub>50</sub> (µg/mL)		IC <sub>50</sub> (µg/mL)	
	24 h	48 h	24 h	48 h
PBMC	ND	ND	ND	ND
K562	345.01 ± 2.53	242.54 ± 2,38	257.49 ± 2.41	223.00 ± 2.34
Jurkat	255.33 ± 2.40	171.45 ± 2,25	256.65 ± 2.40	189.30 ± 2.27

Values are expressed as mean ± SEM (n=3). ND = IC<sub>50</sub> could not be determined.

3. Discussion

The phytochemical constituent profiles of ELSR and ERSR were investigated using HPLC techniques to identify relevant secondary metabolite groups and useful chemical markers. These multi-constituent profile markers are used for quality control of herbal preparations and subsequent validity checks.

An interesting observation in the present study is the high divergence observed in the phytochemical constituents identified between ELSR and ERSR. Besides, the identification for the first time of pharmacologically active metabolites from the roots of *S. rugosa*. This divergence in phytochemical profiles not only underscores the existence of a unique chemical diversity between the distinct parts of the plant but also reflects the intricate specialized metabolic networks characterizing the leaf and root tissues of *S. rugosa*.

The chemical profile of ELSR was characterized predominantly by the accumulation of flavan-3-ol derivatives, notably catechin (L-1). This compound, distinguished by its characteristic UV absorption at 280 nm and a molecular ion  $[M+H]^+$  at  $m/z$  291.0870, was confirmed through comparison with an authentic standard. Proanthocyanidins, including derivatives like epiafzelechin-epiafzelechin (L-4, L-5, L-6) and trihydroxyflavan-epiafzelechin derivatives (L-8, L-9, L-10, L-12), highlight the plant ability to synthesize complex polymeric flavonoids. These compounds were annotated by their molecular masses and distinctive MS/MS fragmentation patterns.

The flavonols, such as Rutin (L-2) and Kaempferol 3-O-rutinoside (L-7), were identified by their UV spectra, MS/MS fragmentation patterns, and comparison with an authentic standard. Flavones like Luteolin (L-11) and its methoxylated derivative, 3-methoxyluteolin (L-13), underscore the plant's ability to synthesize a wide variety of flavonoid structures.

The stilbenoid, 3,3',5,5'-Tetrahydroxy-4-methoxystilbene (L-3), was detected at a low intensity and annotated by its UV absorption and molecular formula. This class has been reported multiple times within the genus [32].

Furthermore, a tetrahydroanthracene derivative (L-16), representing a less common class, was putatively characterized by its molecular weight and MS/MS fragments. It is possibly novel in the literature; due to the absence of standards, we were unable to determine its chemical structure. We recently identified a compound of this class in *S. velutina* [20].

The ERSR extract from the roots displayed a metabolite accumulation distinctly different from that observed in the leaves. Chromones were prominently featured, evidenced by the annotation of obtusichromoneside derivatives (R-2, R-3, R-7) and a chromone derivative (R-7), characterized by their UV spectra and mass fragmentation patterns. These compounds have previously been reported in other species of *Senna* and *Cassia*, a closely related taxon [33].

The analysis also highlighted a significant presence of naphthoquinone and naphthopyrones, with compounds such as 2-methoxystypandrone (R-4), a putative norrubrofusarin gentiobioside (R-8) (a possible novel compound), rubrofusarin gentiobioside (R-10), cassiaside B (R-12), and rubrofusarin-O-glucopyranoside (R-14) demonstrating the root's capability to synthesize complex naphtho-derived structures. These findings are particularly interesting due to the rare occurrence of such compounds in nature and their potential pharmacological applications.

The only compound observed in both the leaves and roots was R-6 (equivalent to L-3 in the leaf), but it demonstrated low chromatographic intensity, consistent with its observation in leaf extracts. Additionally, the detection of Flavanones and their derivatives, including hexahydroxy Flavanonol pentosyl-hexosyl derivatives (R-9, R-13), a putatively annotated hexahydroxy Flavanonol sulfate (R-11), and hexahydroxy-methoxy flavanonol sulfate (R-16), indicates a distinctive flavonoid accumulation pattern in the leaves and roots of *S. rugosa*. Some of these compounds, such as R-11 and R-15 do not match any structures described in the literature, suggesting the possibility that they are novel compounds. The structural diversity within this class highlights the intricate enzymatic processes involved in flavonoid modification.

Seven compounds of the roots remained unidentified (R-1, R-15, R-17, R-18, R-20, R-21, R-22), presenting opportunities for further research. These unknown entities, detected through their unique mass spectra and UV profiles, hint at the unexplored chemical space within *S. rugosa*, suggesting the presence of novel or rare metabolites awaiting discovery.

In summary, this detailed phytochemical investigation significantly enhances our comprehension of the chemical diversity present in *S. rugosa*, underscoring the plant's extensive biosynthetic capacities. It sets a foundation for future research aimed at elucidating the pharmacological properties and ecological significance of these compounds, while also indicating the potential for discovering novel metabolites within this species.

Interestingly, the antioxidant and anticancer activities of most of these compounds identified in ELSR, including their molecular mechanisms have already been proven in several studies. Notably, several *in vitro*, *in vivo*, and clinical studies have shown multiple anticancer actions of epigallocatechin, and a review of their potential therapeutic targets and their role in the therapy of various cancers have been the motive of several studies [34–36]. These include anti-proliferative, pro-

apoptotic, anti-angiogenic, and anti-invasive activities. Another compound found in ELSR is rutin, which has shown ubiquitous pharmacological properties, including antioxidant, anti-inflammatory, antiangiogenic, pro-apoptotic, and antiproliferative activities, all of which may participate in the prevention and treatment of cancer [36].

In the report by Cunha et al., [37] several peaks were observed in the hydroethanolic extract, but only one compound was identified (rutin), making comparison with our results impossible. Thus, we report the presence of different phytochemicals not previously described for this species.

In the ERSR, several pharmacologically important compounds were also identified. Of the twenty-two peaks, 6 could not be identified based on the current technique employed. Among the identified compounds in ERSR were the chromones derivatives, particularly the obtusichromoneside derivatives. Several chromones derivatives have been credited with significant biological and pharmacological activities, including antioxidant, antiviral, anti-inflammatory, antitumor, antimicrobial, among others [38–40]. Duan, et al. [39] presented a comprehensive review of the antitumor activities of naturally occurring chromones. The antitumor mechanisms of these chromones include cytotoxicity (cell-cycle arrest, and cell death, RNA inhibition, and inhibition of signal transducers), antimetastatic, anti-angiogenesis, and immune regulation, among others. Moreover, some have been shown to possess cancer chemoprevention potential. Another equally important secondary metabolites found in ERSR are the naphthoquinones. These groups of naturally occurring bioactive compounds have been the subject of intense research on anticancer and antioxidant activities. [41,42]. The radical scavenging, antioxidant, and anticancer activities of these group of compounds are widely known. These compounds together may act in synergy to account for the antioxidant and prevent oxidative damage to the macromolecules employed in this study.

Genomic mutation of DNA is an essential component in the process of cancer development. Oxidative damage and the consequent modification of DNA bases can lead to point mutations, deletions, insertions, or chromosomal translocations that, in turn, can produce oncogene activation or tumor suppressor gene inactivation [43,44]. In this context, we evaluated the potentials for reactive species sequestration, antioxidant activity, DNA, and protein protective activity against oxidative damage induced by oxidizing agents.

DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA damage (8-hydroxyguanosine) have been noted in various tumors, which strongly implicates such damage in cancer etiology [45]. Furthermore, the increased production of reactive oxygen species (ROS) in various cancers has been shown to play several roles, such as in the activation of pro-tumourigenic signaling, increase in cell survival and proliferation, and drive DNA damage and genetic instability [46–48].

To evaluate the potential protective effect of ELSR and ERSR in mitigating oxidative damage to macromolecules, models of oxidative damage induced by hydroxide peroxide were used and ELSR and ERSR exhibited antioxidant activity, acting in the direct capture of reactive species. Furthermore, we demonstrated that the extracts were able to promote the protection of macromolecules against oxidative damage. These results indicate that the extracts can stabilize reactive species through electron or  $H^+$  donation, preventing nonspecific oxidoreduction reactions that propagate in the intracellular environment [6]. The antioxidant activity of ELSR and ERSR may be related to the presence of biologically active metabolites identified in both extracts. These metabolites are widely recognized for stabilizing reactive oxygen species through hydrogen donation and binding with metal ions [49–51].

In addition to the effect of direct radical scavenging, ELSR and ERSR were able to protect proteins and DNA from oxidative damage. Oxidation of macromolecules by reactive species is an important deleterious effect observed at the cellular level. Protein oxidation promotes the loss of function of receptors, enzymes, and other intracellular proteins, the formation of protein aggregates, and proteolysis.

The protective effect of *S. rugosa* extracts against oxidation of macromolecules suggests that intracellular oxidative damages can be reduced by ingesting the extracts and could present beneficial effects for human health. The intake of natural antioxidants is recognized for promoting a double

beneficial effect on the organism: the protection of healthy cells against the deleterious action of reactive species and anticancer properties. Besides conferring protection to macromolecules, flavonoids, naphthoquinones, among others can regulate the expression of oncogenes and tumor suppressor genes, control cell proliferation, metastasis, and angiogenesis, induce cell cycle arrest, and promote apoptosis in cancer cells [36,41,42,51–53].

Thus, based on the results obtained, ELSR and ERSR may modulate the cellular environment promoting apoptosis activation in K562 and Jurkat cells. From this perspective, further studies are necessary to gain a better understanding of the mechanisms through which the extracts promote the death of leukemic cells.

The search for cytotoxic agents that exhibit selectivity in cancer treatment is increasing [54,55]. In this study, we found that ELSR and ERSR do not affect the viability of healthy cells using PBMC as a model, cells that could be affected during treatment with chemotherapeutic agents during the treatment of leukemias. Since  $IC_{50}$  was not obtained for this cell type, it is possible to infer that ELSR and ERSR exhibit at least 2-3-fold minimal selectivity for leukemic cells. This effect is desirable because the chemotherapeutic agents currently in use are not selective in their actions, causing side effects in the body, such as high toxicity in healthy cells [56]. Thus, we suggest that *S. rugosa* extracts possess potential therapeutic and chemopreventive effects.

Our results demonstrated that the extracts are more effective on the Jurkat cell line, a model of T-cell acute lymphoblastic leukemia. The differences found in our assays among the cell lines utilized might be attributable to mutations in each of the lines investigated. In acute lymphoblastic leukemias, such as Jurkat, silencing of tumor suppressor and pro-apoptotic genes such as p53, PTEN, and BAX is common [15,57]. BCR-ABL1 positive leukemias, as in the K562 cell model, exhibit increased tyrosine kinase activity [13].

## 4. Materials and Methods

### 4.1. Chemicals and Reagents

The reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 2,2'-Azobis(2-methylpropionamidine) dihydrochloride (AAPH), ascorbic acid (AA), bovine serum albumin (BSA), hydroxytoluene butylate (BHT), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), ammonium persulfate and potassium persulfate were purchased from Sigma-Aldrich (São Paulo, Brazil). Electrophoresis reagents were purchased from Bio-Rad Laboratories (São Paulo, Brazil). RPMI-1640 culture medium (Rosewell Park Memorial Institute), antibiotics penicillin, streptomycin, and neomycin, and fetal bovine serum (FBS) were purchased from Gibco/Invitrogen (Minneapolis, USA). The other reagents used in this work were of analytical grade.

### 4.2. Collection and Preparation of Plant Material

The leaves and roots of *S. rugosa* were collected in the city of Dourados, MS, Brazil (22°05'45" S 55°20'46" W), and the permission to access genetic heritage (CTA) was registered in the Heritage SisGen under access code AA6FADF. An exsiccate specimen of the species was deposited in the Herbarium of the Universidade Federal da Grande Dourados with the registration number 4664. Leaves and roots of *S. rugosa* were washed with running water, dried in an oven with air circulation at 36 °C for 10 days, and ground in a knife mill. Leaf (ELSR) and root extracts of *S. rugosa* (ERSR) were prepared from 200 g of leaf or root powder macerated in 95% ethanol at a 1:7 (w/v) ratio at room temperature for 21 days.

Every seven days, the extract was filtered, the solvent was reserved, and a new aliquot of 95% ethanol was added to perform an exhaustive extraction of the compounds. The filtrates were pooled, concentrated in a rotary vacuum evaporator (Gehaka, São Paulo, SP, Brazil) at 37 °C and lyophilized. The yields of the extracts were calculated. The yield of ELSR was 16.61% while that of ERSR was 1%.

## Phytochemical Analyses



The ELSR and ERSR were analyzed in a liquid chromatograph (UFLC, Shimadzu) coupled to a diode array detector (DAD, Shimadzu) and to an electrospray ionization time-of-flight mass spectrometer. (ESI-QTOF-microTOF QII, Bruker Daltonics); positive ionization mode, with detection between 120-1200 Da. The sample was injected into a column C-18 (Kinetex, 2,6  $\mu\text{m}$ , Phenomenex). The chromatographic and mass spectrometric parameters applied were the same used by Castro et al. [19]. We identified the metabolites in the ELSR and ERSR by analyzing their molecular mass, fragmentation patterns, and ultraviolet (UV) absorption spectra, comparing these characteristics with existing literature data. Whenever possible, we confirmed the identities of these compounds through comparative analysis with authentic standards.

### 4.3. Antioxidant Activity Assays

#### 4.3.1. Direct ABTS<sup>•+</sup> Radical Scavenging Assay

The antioxidant capacity of ELSR and ERSR was assessed using ABTS<sup>•+</sup> assay [58]. A volume of 5 mL of ABTS solution (7 mM) and 88  $\mu\text{L}$  of potassium persulfate solution (140 mM) were incubated for 12-16 h at room temperature in the dark. The ABTS<sup>•+</sup> working solution was obtained by diluting the stock solution in distilled water and its absorbance was determined ( $0.70 \pm 0.05$  units) at 734 nm in a spectrophotometer. ELSR or ERSR were prepared at different concentrations (0.1-100  $\mu\text{g/mL}$ ), and 20  $\mu\text{L}$  were mixed with 1,980  $\mu\text{L}$  of the ABTS<sup>•+</sup> working solution. The mixture was incubated for 6 min and absorbance was measured at 734 nm. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as reference antioxidants. A volume of 20  $\mu\text{L}$  of distilled water was used for the determination of the absorbance of the negative control. The percentage of ABTS<sup>•+</sup> radical scavenging was calculated using the following equation 1:

$$\text{Inhibition of ABTS (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (1)$$

Three independent experiments were performed in triplicate and the concentration of the extracts needed for the capture of 50% of the ABTS<sup>•+</sup> radical ( $\text{IC}_{50}$ ) was determined, as well as the reference antioxidants.

#### 4.3.2. Direct DPPH<sup>•</sup> Radical Scavenging Assay

The direct capture of DPPH<sup>•</sup> radical by ELSR and ERSR was evaluated according to Gupta and Gupta [59]. For this, 200  $\mu\text{L}$  of the ELSRs and ERSRs at different concentrations (0.1-100  $\mu\text{g/mL}$ ) were diluted in 1,800  $\mu\text{L}$  of 0.11 mM DPPH<sup>•</sup> solution prepared in 80% ethanol. The mixture was incubated for 30 min at room temperature in the dark. The absorbance at 517 nm was determined. Ascorbic acid and BHT were used as reference antioxidants. A 0.11 mM DPPH<sup>•</sup> solution plus 200  $\mu\text{L}$  of 80% ethanol was used as a negative control. Three independent experiments were performed in triplicate and the percentage of direct DPPH<sup>•</sup> radical scavenging was calculated with the following equation 2:

$$\text{Inhibition of DPPH (\%)} = \frac{1 - \text{Abs sample}}{\text{Abs negative control}} \times 100 \quad (2)$$

The concentration of the extracts required for the capture of 50% of the DPPH<sup>•</sup> radical ( $\text{IC}_{50}$ ) was determined, as well as for the reference antioxidants.

The antioxidant activity assays were performed by diluting the extracts in a 10 mg/mL stock solution with 80% ethanol. From the stock solution, working solutions containing between 5 and 500  $\mu\text{g/mL}$  were prepared in the solvents of each assay.

### 4.4. Protection against Oxidative Damage to the Macromolecules by ELSRs and ERSRs

#### 4.4.1. AAPH-Induced Oxidation of Proteins



The protective effect of the extracts against AAPH-induced protein oxidation was evaluated according to the methodology of Mayo et al. [60], with minor modifications. A 3  $\mu$ L aliquot of BSA (3 mg/mL, prepared in PBS) was pre-incubated with 3  $\mu$ L of the ELSRs or ERSRs at different concentrations (5-500  $\mu$ g/mL) for 30 min at 37 °C. Then, 3  $\mu$ L of AAPH (120 mM, prepared in water) was added to the respective treatments, followed by further incubation for 120 min at 37 °C.

Then, 11  $\mu$ L of sample buffer was added to each tube. The samples were incubated for 5 min at 95 °C and applied to a 12% SDS-PAGE polyacrylamide gel, separated at 200 V on the Mini-PROTEAN Tetra Cell system (Bio-Rad Laboratories). At the end of electrophoresis, the gels were stained, destained, and scanned in a Gel Doc EZ Imager (Bio-Rad Laboratories). The volume of the BSA bands was determined with the help of Image Lab software. Increased volume of the BSA bands was considered oxidative damage. BSA incubated with only AAPH was used as a positive control. The protection conferred by the different concentrations of the extracts was calculated by averaging four gels.

#### 4.4.2. DNA Fragmentation induced by Hydrogen Peroxide ( $H_2O_2$ )

The protective effect of ELSR and ERSR against DNA fragmentation was evaluated using the method described by Kumar and Chattopadhyay [61] with modifications. A 4  $\mu$ L aliquot of plasmid DNA (50 ng/ $\mu$ L) was incubated with 4  $\mu$ L of ELSR or ERSR at different concentrations, diluted in PBS (5-500  $\mu$ g/mL). Then, 4  $\mu$ L of the oxidizing agent  $H_2O_2$  at 30% was added to the respective treatments. The plasmid in the absence of UV light (CT), the plasmid in the presence of UV light (CTUV), the plasmid in the presence of the extracts plus UV light (ELSR or ERSR), and the control plasmid incubated only with hydrogen peroxide ( $H_2O_2$ ) were used as negative control. The effect of other reference antioxidants (rutin and catechin - 150  $\mu$ g/mL) on protecting DNA fragmentation was analyzed. The samples were incubated in a UVT-312 transilluminator at 302 nm at room temperature for 5 minutes. Then, the samples were applied to a 2% agarose gel containing ethidium bromide (10 mg/mL) and subjected to electrophoresis. Images of the gels were scanned using the Gel Doc™ EZ Imager photodocumentator (Bio-Rad Laboratories) and analyzed using Image Lab™ software. The results of 3 gels were expressed as a percentage of DNA fragmentation.

The oxidative damage assays were performed by diluting the extracts in a stock solution containing 0.01% of 80% ethanol supplemented with PBS. From the stock solution, working solutions containing between 5 and 500  $\mu$ g/mL were prepared in the solvents of each assay.

#### 4.5. Cell-Based Assays

##### 4.5.1. Cell Culture

The K562 (Human Chronic Myelogenous Leukemia) and Jurkat (Human Acute Lymphoblastic Leukemia) was grown in suspension in RPMI 1640 media supplemented with 10 nM 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), 24 nM sodium bicarbonate, 10% fetal bovine serum (FBS) and with antibiotic Penicillin-streptomycin (10,000 U/ml), all from Gibco/Invitrogen, Minneapolis, MN, USA. All cells were kept in an incubator with a humidified atmosphere containing 5%  $CO_2$  at 37 °C.

##### 4.5.2. Isolation of Mononuclear Cells from Human Peripheral Blood

The Research Ethics Committee of the Centro Universitário da Grande Dourados (UNIGRAN) approved Blood collection from a healthy nonsmoking donor, process number 123/12. Human peripheral blood mononuclear cells (PBMC) were obtained by centrifugation using Ficoll-Paque-1077 Premium reagent (1.073g/cm<sup>3</sup>). PBMC were suspended in RPMI-1640 medium supplemented with 20% SFB and 1% antibiotic and maintained at 37 °C in a humidified atmosphere containing 5%  $CO_2$ .

##### 4.5.3. Isolation of Mononuclear Cells from Human Peripheral Blood

The potential cytotoxicity of ELSR and ERSR was evaluated with the MTT reagent against leukemic cells (K562 and Jurkat) and PBMC. The K562, Jurkat ( $2 \times 10^4$  cells/well), and PBMC ( $12 \times 10^4$  cells/well) cells were plated in 96-well microplates and treated with different concentrations of the ELSR and ERSR (0-500  $\mu\text{g/mL}$  solubilized in RPMI-1640 medium) for 24 and 48 h. After treatment, the microplates were centrifuged at 1,200 rpm for 5 min and the culture medium was removed. A volume of 100  $\mu\text{L}$  of MTT (0.5 mg/mL, prepared in RPMI-1640 medium) was added to the wells and a 4 h incubation at 37 °C was performed. The plates were centrifuged, had the medium removed, and received 100  $\mu\text{L}$  of dimethyl sulfoxide (DMSO) to solubilize the formazan crystals. A control treatment was performed with 0.1% ethanol, the volume of solvent used for preparing the extracts. The absorbances were determined at 630 nm in a microplate reader (TP READER NM, Thermo Plate) and cell viability was calculated using the following equation 3:

$$\text{Cell viability (\%)} = \frac{\text{Abs treated cells}}{\text{Abs control} - \text{treated cells}} \times 100 \quad (3)$$

The cell viability assay was performed by diluting the extracts in a stock solution containing 0.1% ethanol supplemented with culture medium.

#### 4.6. Statistical Analyzes

Data were presented as mean  $\pm$  standard error of the mean (SEM). Statistically significant differences between groups were analyzed using the Student t-test for comparison between two groups and analysis of variance (ANOVA), followed by Dunnett's test, for comparison of two or more groups, using Prism 7 Software (GraphPad Software, San Diego, CA, USA). Results were considered significant when  $p < 0.05$ .

### 5. Conclusions

Together, we demonstrated that ELSR and ERSR present flavonoid and naphthopyranones derivatives related to the observed antioxidant activities, capable of promoting direct radical scavenging and promoting the protection of macromolecules against oxidative damage. In addition, the ELSR and ERSR exhibited cytotoxicity to human leukemia cell lines without affecting the viability of healthy cells. Our results expand the chemical compounds identified in the leaves and roots of *S. rugosa* and their pharmacological potential in the management conditions related to oxidative stress and leukemia.

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