**Article**

**Phytochemical Insights into *Ficus sur* Extracts and Their Biological Activity**

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**Abstract:** This study focused on the biological evaluation and chemical characterisation of *Ficus sur* Forssk. (*F. sur*) (Family: Moraceae). The methanolic and aqueous extracts' phytochemical profile, antioxidant, and enzyme inhibitory properties were investigated. The aqueous stem bark extract yielded the highest phenolic content (115.51 ± 1.60 mg gallic acid equivalent/g extract), while the methanolic leaves extract possessed the highest flavonoid content (27.47 ± 0.28 mg Rutin equivalent/g extract). In total, 118 compounds were identified in the tested extracts. The methanolic stem bark extract exhibited the most potent radical scavenging potential against 2,2-diphenyl-1 picrylhydrazyl and 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (475.79 ± 6.83 and 804.31 ± 4.52 mg Trolox equivalent/g extract, respectively) and the highest reducing Cu²⁺ capacity (937.86 ± 14.44 mg Trolox equivalent/g extract). The methanolic stem bark extract substantially depressed tyrosinase (69.84 ± 0.35 mg kojic acid equivalent/g extract), α-amylase (0.77 ± 0.01 mmol acarbose equivalent/g extract), acetylcholinesterase and butyrylcholinesterase (2.91 ± 0.07 and 6.56 ± 0.34 mg galantamine equivalent/g extract, respectively) enzymes. *F. sur* extracts were tested for anticancer properties and antiviral activity towards human herpes virus type 1 (HHV-1). Stem bark infusion and methanolic extract showed antineoplastic activity against cervical adenocarcinoma and colon cancer cell lines, whereas leaf methanolic extract exerted moderate antiviral activity towards HHV-1. This investigation yielded important scientific data on *F. sur* which might be used to generate innovative phytopharmaceuticals.

**Keywords:** *Ficus*; natural products; fig; antioxidant; enzymes; phytochemistry; LC-MS; anticancer; natural antivirals

1. **Introduction**

For millennia, humans have centred their lives on plants in an effort to maintain good health and treat common ailments. Even though the usage of plants was based simply on people’s intuitive understanding, owing to a lack of suitable techniques to show plants’ therapeutic potential, humans have accepted the use of many medicinal plants and included them in contemporary pharmacotherapy [1]. The Royal Botanic Gardens at Kew’s Bob Allkin recognised around 28,000 plant species as medicinal plants [2]. Since the eureka moment of the discovery of Taxol, the blockbuster anti-cancer medicine produced from the Pacific yew tree, plants have demonstrated their healing ability [3]. Since then, medicinal...
plants have quickly captured the interest of scientists, resulting in the increased screening of medicinal plants and the use of health-promoting plant extracts in nutrition.

In line with the present worldwide trend, the medicinal plant that this study sought to investigate was *Ficus sur* Forssk. (*F. sur*) (Family: Moraceae). The genus *Ficus* comprises more than 800 species and is generally distributed in tropical and sub-tropical areas [4]. Morphologically, it is a tree that can grow up to 25–30 m tall, with leafy twigs 2–5 mm thick, puberulous, hirtellous, tomentose or hirsute to glabrescent, with the periderm typically not flaking off when dry [5]. *F. sur*, with the common names Cape fig and broom cluster fig, is used to treat a variety of ailments in many countries. Its leaves and roots are used to cure leukommena, leprosy, wounds, oedema, respiratory problems, diarrhoea, sexually transmitted illnesses, tuberculosis, anaemia, epilepsy, rickets, dysentery, male infertility, and gonorrhoea in Sudan and Nigeria [6]. According to ethnobotanical research, *F. sur* is also used to cure swellings [7]. It has long been used in South Africa and other nations to treat renal disorders and as a natural diuretic product [8,9]. In Ethiopia, pulverized fresh *F. sur* leaves combined with water were administered orally as a traditional medicine for urine retention, effectively alleviating the condition by boosting urine production. There is also a traditional belief that the root of this plant may be utilized to treat bladder diseases [10,11]. Despite the reported beneficial effects of members of the genus *Ficus*, several side effects (particularly when eating fruits) have been reported. These include stomach problems, obstructions in the intestine and liver damage [8]. It is necessary not to exceed the recommended dose for the fruits.

The findings of Ayele and co-workers are consistent with the traditional usage of *F. sur* as a diuretic agent. The crude leaf extracts enhanced urine excretion and urinary electrolyte concentrations in a dose-dependent manner [12]. The results of another study indicated that an ethanol extract of *F. sur* has a substantial anticonvulsant effect, validating the traditional use of the plant in the treatment of epilepsies; processes may entail interaction with GABAergic, glycinergic, serotonergic, and glutaminergic system components [13]. The purpose of another study was to see how feeding a mixture of varying amounts of *F. sur* fruits and ground maize grain affected intake, digestibility, growth, and blood profile in Yorkshire pigs [14]. The findings demonstrated that the health of the pigs was better when fed with *F. sur* fruits as creatinine and cholesterol concentrations were lower.

There are reports describing the antiviral potential of different species of *Ficus*. Ethanol extracts from *F. benjamina* leaves inhibited human herpes virus type 1 (HHV-1, HSV-1) and type 2 (HHV-2, HSV-2), and variicella-zoster virus (HHV-3, VZV), while fruit extracts were active only against HHV-3 [15]. *F. carica* latex inhibited caprine herpes virus-1 (CpHV-1) replication in MDBK cells [16], as well as HHV-1, echovirus type 11 (E CV-11) and adenovirus (ADV) replication in VERO cells [17]. Leaf methanol extract from *F. septica* impeded dengue virus (DENV) replication in various infected cell types [18], and *F. religiosa* bark extracts showed activity against human rhinovirus (HRV) and human respiratory syncytial virus (RSV) [19], and HHV-2 [20]. Recently, ethanol extract of *Ficus fistulosa* leaves was reported to show anti-HIV activity [21].

Our aims with this study were to screen methanolic and aqueous leaves and stem bark extracts of *F. sur* for antioxidant and anti-enzymatic activities. Additionally, since the absence of detailed characterisation can markedly limit our understanding of their biological activities, the extracts were subjected to detailed phytochemical profiling. The search for new cholinesterase inhibitors (acetyl- and butyryl-cholinesterase) and carbohydrate digesting inhibitors (α-amylase and α-glucosidase) is now underway and our work has evaluated several extracts of *F. sur* for probable anti-cholinesterase and antidiabetic activities. Since multiple *Ficus* species were shown to possess antiviral activity, we have undertaken the attempt to evaluate the anti-HHV-1 activity of extracts obtained from *Ficus sur*. Furthermore, we have evaluated the anticancer potential of this plant species against cervical adenocarcinoma and colon cancer cell lines. We hope that the information offered here will assist in bridging a research gap and, as a result, open up new research opportunities, notably in the production of medicinal bioproducts.
2. Results and Discussion

2.1. Bioactive Compounds

The polarity of the solute of interest was considered in choosing the solvents used to extract bioactive compounds from plants because a solute with equivalent polarity to the solvent will be sufficiently dissolved according to the law of similarity and intermiscibility [22,23]. In this study, polyphenolic compounds, such as phenolic compounds and flavonoids, were quantified from the methanolic and aqueous extracts of *F. sur*. The results are presented in Table 1.

Among the samples studied, the aqueous extract obtained from the stem bark yielded the highest amount of phenolics (115.51 ± 1.60 mg GAE/g), while the methanolic extract prepared from leaves had the highest flavonoid content (27.47 ± 0.28 mg RE/g). LC-ESI-QTOF-MS/MS analysis enabled the chemical characterization of all studied extracts. In total, 118 compounds were described (Table 2, Figure 1). It was observed that the leaf extracts contained more compounds compared to the stem bark extracts. Phenolic acids (such as quinic, citric, dihydroxybenzoic, 3-O-cafeoylquinic, and 5-O-cafeoylquinic acid), quercetin glycosides (quercetin-O-di-rhamnosyl-glucoside/galactoside, quercetin-O-glucoside, quercetin-O-pentoside (arabinoside), quercetin-O-glucuronide), and fatty acids (hydroxy octadecatrienoic acid, palmitic acid derivative) were present in almost all extracts. The methanolic extract from leaves contained mainly phenolic acids and their derivatives, esters of phenolic acids and flavonoids, and flavonoid glycosides (esters of kaempferol and quercetin). Hydroxycoumarin and methyl gallate were present only in this extract. Hydroxycaffeoylquinic, glucogallic, 2-isopropylmalic, tartaric, coumaric, ferulic acids and their esters were characteristic for leaf infusion. The methanolic extract from the stem bark was abundant in tannins, represented by catechins and procyanidins. In the stem bark infusion, apigenin, luteolin, kaempferol, quercetin and their conjugates with one or more sugar moieties dominated. The majority of the detected secondary metabolites were typical for previously studied *Ficus* species: *F. lyrata*, *F. benghalensis*, *F. benjamina*, *F. mysorensis*, *F. Afzelii*, *F. pyriformis*, *F. racemose*, *F. lutea*, *F. auriculata*, *F. trigonata*, *F. spragueana*, *F. microcarpa* var. *nitida*; *F. virens* and *F. religiosa* for which the presence of flavonoids, flavonolignans, anthocyanins and hydroxycinnamic acids derivatives was reported [24,25].

### Table 1. Total phenolic and flavonoid contents of *Ficus sur* extracts.

| Parts         | Solvents | TPC (mg GAE/g) | TFC (mg RE/g) |
|---------------|----------|----------------|---------------|
| Leaves        | MeOH     | 58.46 ± 0.28 c | 27.47 ± 0.28 a |
|               | Infusion | 51.77 ± 0.77 d | 16.65 ± 0.18 b |
| Stem barks    | MeOH     | 109.79 ± 2.19 b| 2.54 ± 0.10 c |
|               | Infusion | 115.51 ± 1.60 a| 1.13 ± 0.11 d |

Values are reported as mean ± SD of three parallel measurements. GAE: Gallic acid equivalents; RE: Rutin equivalents. Different letters in the same column indicate significant differences in the tested extracts (p < 0.05).

### Table 2. Compounds identified in the studied extracts.

| Comp. No | Tentative Identification | R Time | Molecular Mass | [M – H] | Fragment Ions (m/z) | Extracts |
|----------|--------------------------|--------|----------------|---------|---------------------|----------|
| 1        | Quinic acid              | 1.91   | 192.0507       | 191.0507| 173.0464; 111.0437; 93.0318; 85.0262 | 1,2,3    |
| 2        | Citric acid              | 2.33   | 192.0180       | 191.0180| 111.0035; 87.0052   | 1,2,3,4  |
| 3        | Caffeic acid derivative  | 3.40   | 242.0302       | 241.0302| 179.0273; 153.0497; 135.0406; 123.0407; 109.0230 | 4        |
| 4        | Quinic acid derivative   | 3.46   | 534.1621       | 533.1621| 337.0845; 191.0508   | 2        |
| 5        | Quinic acid derivative   | 4.04   | 406.0968       | 405.0968| 213.0351; 191.0511   | 4        |
| Comp. No | Tentative Identification | R Time | Molecular Mass | [M – H]− | Fragment Ions (m/z) | Extracts |
|-----------|-------------------------|--------|----------------|----------|---------------------|---------|
| 6         | 4-Hydroxy-2-(hydroxymethyl)benzoic acid | 4.09   | 168.0307       | 167.0307 | 149.0254; 123.0429  | 3       |
| 7         | Dihydroxybenzoic acid glucoside derivative | 4.13   | 532.0929       | 531.0929 | 353.0781; 315.0642; 153.0155; 96.9570 | 2       |
| 8         | Quinic acid derivative | 4.22   | 470.0574       | 469.0574 | 435.1422; 371.0962; 191.0423 | 2       |
| 9         | Hydroxycaffeoyl-quinic acid | 4.76   | 372.0900       | 371.0900 | 353.0840; 197.0360; 191.0533; 179.0312; 173.0431; 135.0409 | 2       |
| 10        | Glucogalic acid/Glucosyl gallate | 6.32   | 332.0607       | 331.0607 | 169.0124; 151.0003; 125.0211 | 2       |
| 11        | Dihydroxybenzoic acid glucoside derivative | 6.49   | 436.0075       | 435.0075 | 315.0710; 153.0568 | 1       |
| 12        | Dihydro-caffeoyl-quinic acid | 6.56   | 356.0959       | 355.0959 | 191.0522; 181.0167; 173.0451; 137.0164; 111.0044 | 2       |
| 13        | Hydroxybenzoic acid derivative | 6.77   | 432.1224       | 431.1124 | 137.0243; 93.0383 | 3       |
| 14        | Caffeoyl-hydroxybenzoic acid | 7.23   | 300.0717       | 299.0717 | 239.0562; 179.0356; 137.0228 | 4       |
| 15        | Dihydroxybenzoic acid glucoside isomer 1 | 7.48   | 316.0628       | 315.0628 | 153.0134; 152.0105; 152.0105; 121.0129 | 1,2     |
| 16        | Dihydroxybenzoic acid | 7.59   | 154.0143       | 153.0143 | 109.0302; 108.0225 | 1,2,3,4 |
| 17        | Coumaric acid-hexoside-pentoside | 8.48   | 458.0892       | 457.0892 | 325.0865; 163.0347 | 2       |
| 18        | Dihydroxybenzoic acid glucoside isomer 2 | 8.51   | 316.0660       | 315.0660 | 153.0147; 109.0258 | 2       |
| 19        | 3-Hydroxy-4-methoxymandelate glucoside | 8.86   | 360.0924       | 359.0924 | 197.0449; 182.0215; 153.0557; 138.0321; 123.0129 | 3       |
| 20        | Caffeic acid derivative hexoside | 9.06   | 376.0601       | 375.0601 | 341.1069; 213.0650; 201.0144; 179.0316; 135.0409 | 1       |
| 21        | Quinic acid derivative | 9.08   | 372.0868       | 371.0868 | 251.0544; 191.052; 167.0327 | 1       |
| 22        | 2-Isopropylmalic acid | 9.19   | 176.0571       | 175.0571 | 157.0486; 115.0371; 85.0638 | 2       |
| 23        | Hydroxybenzoic acid | 9.29   | 138.0208       | 137.0208 | 108.0231 | 3,4     |
| 24        | Unidentified | 9.39   | 376.1236       | 375.1236 | 312.0725; 169.0827; 151.0726 | 4       |
| 25        | Hydroxybenzoic acid 4-O-glucoside | 10.17  | 300.0669       | 299.0669 | 137.0203; 93.0315 | 1,2     |
| 26        | 3-O-Caffeoylquinic acid | 10.36  | 354.0782       | 353.0782 | 191.0578; 179.0370; 161.0243; 135.0461 | 1,2,3   |
| 27        | Dihydroxybenzoic acid glucoside isomer 3 | 10.69  | 316.0660       | 315.0660 | 153.0144; 109.0258 | 2       |
| 28        | Caffeic acid glucoside | 10.76  | 342.0825       | 341.0825 | 179.0357; 161.0246; 135.0457 | 4       |
| 29        | Dihydroxybenzoic acid O-glucoside-pentoside | 10.84  | 448.1382       | 447.1382 | 315.1063; 153.0548; 109.0301; 108.0249 | 3       |
| 30        | Hydroxyconarinar | 10.93  | 340.0715       | 339.0715 | 177.0178 | 1       |
| 31        | Methyl gallate | 11.02  | 184.0234       | 183.0234 | 168.0071; 124.0155; 78.0128 | 1       |
| 32        | Hydroxybenzoic acid | 11.13  | 138.0210       | 137.0210 | 109.0209; 108.0308; 93.0345 | 4       |
| 33        | Glucogalic acid/Glucosyl gallate | 11.50  | 332.0607       | 331.0607 | 169.0113; 168.0047; 125.0225 | 1,2,4   |
| 34        | 3-O-p-Coumaroylquinic acid | 11.30  | 338.0840       | 337.0840 | 191.0540; 163.0384 | 1,2     |
| 35        | Procyanidin B (dimer of (epi)catechin) | 11.62  | 578.1270       | 577.1270 | 451.1080; 425.0892; 407.0834; 289.0754; 245.0811; 125.0259 | 3       |
| 36        | Caffeic and coumaric acid derivative | 12.20  | 542.1494       | 541.1494 | 523.1466; 475.1519; 361.0900; 235.0490; 215.0901; 179.0358; 163.0398; 137.0249 | 4       |
| 37        | 1-O-Coumaroylquinic acid | 12.28  | 338.0840       | 337.0840 | 191.0509; 173.0417; 163.0356 | 1,2     |
| Comp. No | Tentative Identification | R Time | Molecular Mass (m/z) | [M − H]− (m/z) | Fragment Ions (m/z) | Extracts |
|----------|-------------------------|--------|---------------------|----------------|---------------------|----------|
| 38       | Glucogallic acid tartaric ester | 12.33  | 464.1040            | 463.1040       | 331.0608; 169.0147; 168.0037; 149.9937; 125.0212 | 3        |
| 39       | Caffeic acid glucoside       | 12.34  | 342.0825            | 341.0825       | 179.0344; 161.0230; 135.0440 | 4        |
| 40       | Gallic acid glucoside derivative | 12.55 | 412.0173            | 411.0173       | 367.355; 331.0632; 240.9965; 169.0991; 125.0157 | 2        |
| 41       | Caffeic acid derivative hexoside | 12.63 | 542.1494            | 541.1494       | 379.0983; 179.0323; 161.0220; 135.0425 | 4        |
| 42       | Methyl gallate hexoside-pentoside | 12.70 | 448.1542            | 447.1542       | 345.1151; 183.0660; 168.0382; 161.0437 | 3        |
| 38       | (Epi)gallocatechin           | 13.18  | 306.0612            | 305.0612       | 287.0562; 269.0489; 219.0678; 195.0310; 179.0321; 161.0246; 137.0237; 125.0235 | 3        |
| 44       | 5-O-Caffeoylquinic acid      | 13.27  | 354.0811            | 353.0811       | 191.0521; 161.0214; 135.0406 | 1,2,3    |
| 45       | Dihydroxycoumarin            | 13.47  | 178.0152            | 177.0152       | 161.0960; 133.0279; 105.0338 | 4        |
| 46       | 3-O-Feruloylquinic acid      | 13.66  | 368.0964            | 367.0964       | 193.0481; 173.0430; 134.0355 | 2        |
| 47       | 4-O-Caffeoylquinic acid      | 14.32  | 354.0811            | 353.0811       | 191.0516; 179.0306; 173.0412; 135.0414 | 2        |
| 48       | Caffeic acid                 | 14.41  | 180.0293            | 179.0293       | 135.0451 | 1,4        |
| 49       | Coumaroyltartaric acid isomer 1 | 14.68 | 296.0404            | 295.0404       | 163.0357; 149.0051; 130.9957; 119.0460 | 2        |
| 50       | Tartaric acid                | 14.94  | 150.0055            | 149.0055       | 130.9997; 102.9993; 87.0065 | 2        |
| 51       | 4-O-Methylgallocatechin      | 15.25  | 320.0763            | 319.0763       | 287.0497; 243.0251; 197.0451; 161.0242; 125.0233 | 3        |
| 52       | Caffeic and hydroxycinnamic acid derivative | 15.39 | 380.0995            | 379.0995       | 369.0971; 251.0590; 217.0658; 179.0345; 161.0261; 135.0426 | 4        |
| 53       | Coumaric acid hexoside derivative | 15.49 | 442.0969            | 441.0969       | 325.0912; 163.0378; 119.0507 | 2        |
| 54       | Coumaric acid                | 15.76  | 164.0361            | 163.0361       | 119.0483 | 2        |
| 55       | Coumaroyltartaric acid isomer 2 | 15.82 | 296.0404            | 295.0404       | 163.0363; 149.0056; 130.9944; 119.0472 | 2        |
| 56       | B-type procyanidin trimer    | 16.03  | 866.1873            | 865.1873       | 739.1731; 713.1525; 577.1396; 425.0996; 287.0524; 245.0479; 125.0216 | 3        |
| 57       | Caffeic acid pentoside       | 16.15  | 312.0340            | 311.0340       | 179.0356; 135.0380 | 2        |
| 58       | 4-O-p-Coumaroylquinic acid  | 16.33  | 338.0871            | 337.0871       | 191.0520; 173.0423; 163.0363 | 1,2      |
| 59       | Procyanidin B (dimer of (epi)catechin) | 16.54 | 578.1226            | 577.1226       | 451.0947; 425.0842; 407.0791; 289.0774; 245.0500 | 1,3      |
| 60       | Caffeic acid and hydroxycoumarin derivative | 16.93 | 458.1282            | 457.1282       | 383.0989; 221.0481; 179.0362; 161.0235; 135.0433; 133.0275; 117.0335 | 4        |
| 61       | (Epi)catechin derivative     | 17.11  | 326.0425            | 325.0425       | 289.0667; 245.0790; 205.0449; 125.0275 | 3        |
| 62       | Sinapoyl-ferulate            | 17.22  | 400.0876            | 399.0876       | 223.0469; 205.0374; 193.0429; 129.0150; 111.0050; 85.0271 | 2        |
| 63       | (Epi)catechin                | 17.26  | 290.0637            | 289.0637       | 245.0785; 205.0476; 187.0356; 179.0295; 125.0258 | 1,3      |
| 64       | Ferulic acid pentoside       | 18.03  | 326.0500            | 325.0500       | 193.0474; 178.0238; 134.0347 | 2        |
| 65       | Caffeoylshikimic acid        | 18.03  | 336.0713            | 335.0713       | 179.0325; 173.0429; 161.0208; 135.0420 | 2        |
| 66       | 5-O-p-Coumaroylquinic acid  | 18.50  | 338.0871            | 337.0871       | 191.0514; 173.0417; 163.0356 | 1,2      |
| Comp. No | Tentative Identification | R Time (min) | Molecular Mass | [M – H]+ | Fragment Ions (m/z) | Extracts |
|----------|-------------------------|-------------|----------------|----------|---------------------|---------|
| 67       | Unidentified            | 18.67       | 578.1268       | 577.1268 | 541.1482; 379.0918; 179.0298; 161.0208; 135.0406 | 4       |
| 68       | Caffeoylshikimic acid isomer | 19.17       | 336.0713       | 335.0713 | 179.0302; 173.0411; 161.0200; 155.0291; 135.0415 | 1, 2    |
| 69       | B-type procyanidin trimer | 19.20       | 866.1873       | 865.1873 | 739.1553; 713.1344; 577.1240; 425.0756; 287.0531; 245.0383; 125.0183 | 3       |
| 70       | 4-O-Feruloylquinic acid | 19.75       | 368.0972       | 367.0972 | 367.0972; 191.0531; 173.0423 | 1, 2    |
| 71       | Caffeoylmalic acid       | 20.07       | 296.0416       | 295.0416 | 179.0315; 133.115; 115.0010 | 1, 2    |
| 72       | Procyanidin B (dimer of (epi)catechin) derivative | 20.07       | 880.1656       | 879.1656 | 727.1275; 577.1073; 439.0638; 407.0538; 287.0468; 245.0207 | 3       |
| 73       | B-type procyanidin trimer | 20.38       | 866.1873       | 865.1873 | 739.1553; 713.1344; 577.1240; 425.0756; 287.0531; 245.0383; 125.0183 | 3       |
| 74       | (Epi)catechin-(epi)gallocatechin | 20.55       | 594.1023       | 593.1023 | 575.1061; 467.1034; 441.0792; 423.0659; 305.0549; 287.0417; 245.0207 | 3       |
| 75       | Procyanidin B (dimer of (epi)catechin) derivative | 20.76       | 721.6486       | 720.6486 | 644.1217; 577.1295; 289.0645; 289.0417; 245.0207 | 3       |
| 76       | Quercetin-O-d-glucoside | 21.27       | 626.1342       | 625.1342 | 463.0867; 301.0301; 300.0238; 178.9961; 151.0005 | 4       |
| 77       | Quercetin-O-d-rhamnosyl-glucoside/galactoside | 21.28       | 756.1880       | 755.1880 | 609.1432; 300.0275; 271.0254; 178.9947; 151.0009 | 1, 2, 3 |
| 78       | Unidentified            | 21.74       | 536.1387       | 535.1387 | 491.1498; 323.0715; 281.0600; 179.0314; 161.0215 | 4       |
| 79       | Procyanidin dimer monoglycoside | 22.18       | 740.1601       | 739.1601 | 587.1146; 459.0606; 449.0773; 435.0625; 289.062; 245.0766; 125.0124 | 3       |
| 80       | 5,8-Dihydroxy-7-methoxyflavone-O-glucoside-rhamnoside | 22.22       | 592.1811       | 591.1811; | 445.1129; 325.0735; 297.0404; 293.0638; 282.0504 | 1       |
| 81       | Quercetin-3-O-arabino-glucoside | 22.24       | 596.1230       | 595.1230 | 301.0279; 300.0229; 271.0219; 255.0251; 178.9902 | 4       |
| 82       | Rutin                  | 23.06       | 610.1377       | 609.1377 | 301.0285; 300.0216; 271.0215; 178.9902; 150.9995 | 3, 4    |
| 83       | Procyanidin B (dimer of (epi)catechin) | 23.25       | 578.1270       | 577.1270 | 451.0997; 425.0849; 407.0722; 289.0671; 125.0228 | 3       |
| 84       | Rutin-O-(p-coumaroyl) malate | 23.32       | 890.1862       | 889.1862 | 609.1326; 300.0192; 271.0182; 178.9951; 150.9965; 133.0093 | 1, 2    |
| 85       | Quercetin derivative hexoside | 23.85       | 566.1131       | 565.1131 | 403.1489; 301.0262; 271.0208; 255.0250; 178.9927; 150.9985 | 4       |
| 86       | Quercetin-O-glucoside   | 23.86       | 464.0778       | 463.0778 | 300.0028; 271.0183; 178.9940; 150.9981 | 1, 2, 3 |

Table 2. Cont.
### Table 2. Cont.

| Comp. No | Tentative Identification                  | R Time | Molecular Mass  | [M – H]− | Fragment Ions (m/z)                                                                 | Extracts |
|----------|------------------------------------------|--------|----------------|----------|-----------------------------------------------------------------------------------|----------|
| 91       | Quercetin-O-pentoside (arabinoside)      | 24.75  | 434.0683       | 433.0686 | 301.0267; 300.0213; 271.0193; 255.0248; 227.0281; 150.9994                         | 1, 2, 3  |
| 92       | Kaempferol-C-di-hexoside-O-hexoside      | 24.85  | 772.1694       | 771.1694 | 609.1382; 485.1224; 429.0806; 383.0925; 323.0716; 285.0338; 255.0239; 227.0213; 161.0214; 133.0241 | 4        |
| 93       | Luteolin-O-glucuronide                   | 25.13  | 462.0664       | 461.0664 | 285.0343; 175.0245; 133.0246                                                      | 4        |
| 94       | Kaempferol-O-glucoside                   | 25.18  | 448.0832       | 447.0832 | 284.0261; 255.0237; 227.0277; 150.9974                                           | 1        |
| 95       | Quercetin-O-(caffeoyl-di-glucoside)      | 25.44  | 788.1650       | 787.1650 | 625.1372; 461.0677; 387.1494; 301.0286; 300.0232; 179.0375; 161.0161              | 4        |
| 96       | Quercetin-O-glucuronide                  | 25.71  | 478.0832       | 477.0832 | 301.0285; 271.0556; 179.9914; 150.9982                                           | 1, 2, 3  |
| 97       | Quercetin-O-arabinoside-di-glucoside     | 25.92  | 758.1530       | 757.1530 | 595.1207; 463.0830; 301.0292; 300.0215; 178.9926; 150.9968                       | 4        |
| 98       | Quercetin-O-glucoside-arabinoside-glucuronide | 26.21  | 772.1694       | 771.1694 | 595.1243; 301.0275; 300.0228; 271.0201; 178.9963; 150.9999                       | 4        |
| 99       | Kaempferol-O-di-pentoside                | 26.40  | 550.1142       | 549.1142 | 417.0841; 285.0453                                                               | 1, 3     |
| 100      | Kaempferol-O-pentoside                   | 26.42  | 418.0743       | 417.0743 | 284.0376; 285.0421                                                               | 1, 2     |
| 101      | Di-caffeoyl-dihydroxybenzoic acid        | 26.60  | 478.0983       | 477.0983 | 433.1033; 315.0667; 179.0321; 161.0204; 153.0140; 152.0098; 135.0443; 109.0295; 108.0158 | 4        |
| 102      | (Epi)-alzelechin-7-O-glucoside           | 26.82  | 436.1199       | 435.1199 | 345.0936; 273.0730; 167.0336                                                 | 1        |
| 103      | Kaempferol-O-(caffeoyl-arabinoside-glucoside) | 27.09  | 742.1608       | 741.1608 | 579.1326; 455.1050; 429.0733; 285.0349; 284.0270; 255.0312; 227.0223; 179.0308; 161.0221; 135.0376 | 4        |
| 104      | Kaempferol-O-rhamnoside                  | 27.88  | 432.0906       | 431.0906 | 285.0341; 284.0317; 255.0287; 227.0334                                            | 1, 2     |
| 105      | Quercetin-O-glucoside-arabinoside-glucuronide | 27.99  | 772.1694       | 771.1694 | 595.1228; 301.0258; 300.0216; 271.0204; 255.0185; 178.9945; 150.9824              | 4        |
| 106      | Kaempferol-O-arabinoside-glucuronide     | 29.28  | 726.1658       | 725.1658 | 579.1227; 285.0346; 284.0272; 255.0222; 227.0298                                | 4        |
| 107      | Luteolin                                | 31.09  | 286.0364       | 285.0364 | 175.0368; 133.0300                                                               | 4        |
| 108      | Trihydroxy-octadecadienoic acid          | 32.36  | 328.2116       | 327.2116 | 291.1989; 229.1460; 211.1336; 171.1031                                           | 2, 4     |
| 109      | Unidentified                            | 32.36  | 396.1960       | 395.1960 | 349.2045; 327.2269; 251.1307; 233.1170; 193.0888; 171.1032                        | 1, 3     |
| 110      | Trihydroxy-octadecenoic acid             | 33.91  | 330.2292       | 329.2292 | 311.2203; 293.1239; 229.1450; 211.1334; 171.1011                                | 4        |
| 111      | 12-oxo-10E-dodecenoic acid               | 34.01  | 228.1222       | 227.1222 | 209.1179; 183.1395; 165.1298                                                  | 1, 2     |
| 112      | Apigenin                                | 34.28  | 270.0417       | 269.0417 | 227.0349; 151.0027; 117.0349; 107.0126                                            | 4        |
| 113      | Trihydroxy-octadecenoic acid derivative  | 35.26  | 444.1493       | 443.1493 | 329.2280; 309.1244; 293.1891                                                   | 4        |
| 114      | 12-oxo-10E-dodecenoic acid derivative    | 43.89  | 722.3519       | 721.3519 | 675.3594; 397.1348; 227.2159                                                   | 1, 4     |
| 115      | Hydroxyl octadecatrienoic acid           | 47.40  | 294.2048       | 293.2048 | 275.2015; 224.1403; 195.1388                                                  | 1, 2, 4  |
| 116      | Palmitic acid derivative                 | 47.50  | 700.3671       | 699.3671 | 653.7889; 397.1369; 255.2329                                                   | 1        |
Table 2. Cont.

| Comp. No | Tentative Identification          | R Time | Molecular Mass  | [M − H]− | Fragment Ions (m/z) | Extracts |
|----------|----------------------------------|--------|----------------|----------|--------------------|----------|
| 117      | Palmitic acid derivative         | 48.36  | 541.3192       | 540.3192 | 480.3059; 255.2310  | 1,3,4    |
| 118      | Hydroxy octadecadienoic acid derivative | 49.62  | 366.2055       | 365.2055 | 317.2080; 295.2254; 277.2110 | 1,2      |

1 Ficus sur leaves-MeOH; 2 Ficus sur leaves-infusion; 3 Ficus sur stem bark-MeOH; 4 Ficus sur stem bark -infusion. The identification was supported by the following sources [24–37].

Figure 1. Base peak chromatograms of studied extracts. 1 Ficus sur leaves-MeOH; 2 Ficus sur leaves-infusion; 3 Ficus sur stem bark -MeOH; 4 Ficus sur stem bark -infusion.

2.2. Antioxidant Effects

The role of oxidative stress in the initiation and progression of human diseases supports the systemic antioxidant assessment of plant extracts under investigation. Antioxidants can perform various functions, including hydrogen atom transfer, single electron transfer, and transition metal chelation [38]. In this study, a battery of antioxidant assays was used to obtain a comprehensive understanding of the antioxidant activities of the prepared extracts of F. sur. The assays were: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric ion reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), metal-chelating and total antioxidant capacity (phosphomolybdenum). As previously discussed, each test has its own set of advantages and disadvantages [38]. The results are given in Table 3.

Overall, irrespective of the type of extraction solvents used, stem bark extracts demonstrated substantially higher antioxidant activities with DPPH, ABTS, CUPRAC, FRAP, and phosphomolybdenum. For instance, methanolic stem bark extract exhibited the highest DPPH radical scavenging activities (475.79 ± 6.83 mg TE/g). The ABTS assay showed that both methanolic (804.31 ± 4.52 mg TE/g) and aqueous stem bark (804.91 ± 5.45 mg TE/g) extracts demonstrated remarkably high activities. ABTS can function with lipophilic and hydrophilic molecules, but DPPH can only be solubilized in organic environments [39]. Our findings confirm the findings of Kim et al. [39]. For example, the DPPH test identified the
molecules as the most active, while ABTS identified both methanolic and aqueous extracts as effective ABTS scavengers.

The antioxidant capacity of the extracts was further evaluated in terms of power reduction using the CUPRAC and FRAP tests. Several variables influence antioxidants’ reducing potential, including their ionization potentials, the spin distribution of radical cations, and the bond dissociation energy of the phenolic O-H bond [40]. From Table 3, it can be seen that the methanolic stem bark extract possessed the most potent Cu\(^{2+}\) reducing potential (938.86 ± 14.44 mg TE/g) while the aqueous stem bark extract (614.33 ± 2.79 mg TE/g) was the most robust Fe\(^{3+}\) reducer.

Secondary metabolites are known to have powerful antioxidant properties due to their ability to provide electrons and because they chelate transition metals [41]. Data shown in Table 3 show that the aqueous leaves extract exhibited the highest chelating abilities (22.95 ± 0.20 mg EDTAE/g) while the methanolic stem bark extract displayed the lowest activity (4.62 ± 0.64 mg EDTAE/g). The prepared samples were also tested for their total antioxidant capacity (phosphomolybdenum assay). The latter test is based on antioxidants reducing Mo (VI) to Mo (V), resulting in the formation of a green complex in acidic conditions [42]. The aqueous stem bark extract showed the highest capacity (5.05 ± 0.05 mmol TE/g). It is noteworthy that the stem bark extracts showed stronger antioxidant activity than the leaf extracts for all assays, except the metal-chelating assay. Consequently, it can be said that the antioxidant activity of the active samples could be associated with the presence of bioactive compounds.

### Table 3. Antioxidant properties of *Ficus sur* extracts.

| Parts   | Solvents | DPPH (mg TE/g) | ABTS (mg TE/g) | CUPRAC (mg TE/g) | FRAP (mg TE/g) | PBD (mmol TE/g) | MCA (mg EDTAE/g) |
|---------|----------|----------------|----------------|------------------|----------------|----------------|------------------|
| Leaves  | MeOH     | 48.66 ± 0.04 c | 81.41 ± 0.05 b | 160.80 ± 1.55 c  | 108.50 ± 2.04 c | 1.65 ± 0.11 b  | 7.88 ± 0.99 c     |
|         | Infusion | 44.22 ± 0.06 c | 72.32 ± 1.69 b | 147.58 ± 1.59 c  | 77.28 ± 0.25 d  | 1.65 ± 0.08 b  | 22.95 ± 0.20 a    |
| Stem barks | MeOH   | 475.79 ± 6.83 a | 804.31 ± 4.52 a | 937.86 ± 14.44 a | 523.17 ± 2.92 b | 5.00 ± 0.30 a  | 4.62 ± 0.64 d     |
|         | Infusion | 463.58 ± 1.17 b | 804.91 ± 5.45 a | 910.68 ± 12.14 b | 614.33 ± 2.79 a | 5.05 ± 0.05 a  | 13.22 ± 0.18 b    |

Values are reported as mean ± SD of three parallel measurements. TE: Trolox equivalents; EDTAE: EDTA equivalents. Different letters in the same column indicate significant differences in the tested extracts (p < 0.05).

### 2.3. Enzymatic Inhibitory Activities

In the present study, the ability of *F. sur* extracts to modulate the activity of enzymes related to Alzheimer’s disease [acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)], diabetes type 2 (α-amylase and α-glucosidase), and skin hyperpigmentation (tyrosinase) was investigated. The results are presented in Table 4.

Because enzymes in the human body contribute to the genesis of disease, inhibiting these enzymes can be advantageous in health care. Cholinesterase inhibitors, for example, are drugs that prevent the breakdown of acetylcholine, a neurotransmitter in the central nervous system that, when present in excessive concentrations, can cause neurodegenerative diseases, such as Alzheimer’s and Parkinson’s disease [43]. Our study explored the anti-cholinesterase activity in various extracts of *F. sur*. High anti-AChE and anti-BChE activities were recorded with the methanolic stem bark extract (2.91 ± 0.07 and 6.56 ± 0.34 mg GALAE/g, respectively). However, the aqueous leaves extract was inactive against AChE and BChE.

Inhibitors of α-amylase and α-glucosidase diminish carbohydrate digestion in the small intestine and, as a result, lower postprandial blood glucose levels, making them an essential therapy option for type II diabetes patients [44]. The methanolic stem bark extract of *F. sur* was observed to substantially depress α-amylase (0.77 ± 0.01 mmol ACAE/g) but was found to be inactive against α-glucosidase. Instead, the methanolic leaves extract showed high anti-glucosidase activity (3.98 ± 0.03 mmol ACAE/g).

Tyrosinase inhibitors help to protect the skin and prevent hyperpigmentation. They are strongly promoted by the pharmaceutical and cosmetics industries [45]. The methanolic
stem bark extract displayed the strongest anti-tyrosinase activity (69.84 ± 0.35 mg KAE/g), while the aqueous leaves extract showed the lowest activity (0.35 ± 0.08 mg KAE/g). It is noteworthy that the methanolic stem bark extract showed the highest activity against four enzymes, namely AChE, BChE, tyrosinase, and α-amylase, although, the extract did not show the highest TFC and TPC.

Table 4. Enzyme inhibitory properties of *Ficus sur* extracts.

| Parts          | Solvents | AChE (mg GALAE/g) | BChE (mg GALAE/g) | Tyrosinase (mg KAE/g) | Amylase (mmol ACAE/g) | Glucosidase (mmol ACAE/g) |
|----------------|----------|-------------------|-------------------|----------------------|-----------------------|--------------------------|
| Leaves         | MeOH     | 2.11 ± 0.24       | 2.31 ± 0.21       | 68.12 ± 0.47         | 0.61 ± 0.01           | 3.98 ± 0.03              |
|                | Infusion | na                | na                | 0.35 ± 0.08          | 0.13 ± 0.01           | 3.90 ± 0.01              |
| Stem barks     | MeOH     | 2.91 ± 0.07       | 6.56 ± 0.34       | 69.84 ± 0.35         | 0.77 ± 0.01           | na                       |
|                | Infusion | 1.88 ± 0.11       | na                | 51.55 ± 0.24         | 0.74 ± 0.02           | na                       |

Values are reported as mean ± SD of three parallel measurements. GALAE: Galantamine equivalents; KAE: Kojic acid equivalents; ACAE: Acarbose equivalents; na: not active. Different letters in the same column indicate significant differences in the tested extracts (*p* < 0.05).

2.4. Cytotoxicity Evaluation

Cytotoxicity evaluation revealed that the infusion and methanolic extract from *Ficus sur* leaves exerted low toxicity on normal kidney fibroblasts (VERO); the exact CC$_{50}$ values could not be evaluated because they were above the tested concentration range (Table 5). Stem bark extracts showed a similar effect on VERO cells. Selective toxicity towards HeLa cancer cells was observed for *Ficus sur* leaves methanolic extract (FLM) and infusion (FLI) with SI of >3.62 and >2.36. In contrast, in the case of RKO, only FLM showed selective toxicity (SI > 3.13). Significant antineoplastic activity towards both cancer cell lines was observed (Figure 2) for *Ficus sur* stem bark methanolic extract (FSBM) and infusion (FSBI) with CC$_{50}$ values ranging from 36.8 to 56.12 µg/mL. The anticancer selectivity of FSBM and FSBI towards HeLa cells was 7.1 and 9.24, respectively, whereas against RKO, it was found to be 5.37 and 7.01, respectively. Multiple studies describe the anticancer potential of *Ficus* spp, ex. *Ficus carica* [46,47], *Ficus salicifolia* [46], *Ficus religiosa* [48], *Ficus beecheyana* [49], *Ficus pandurata* H [50] and *Ficus exasperata* (Vahl) [51], against various cancer cell lines, however, to the best of our knowledge, this is the first report showing *Ficus sur* stem bark extracts as a possible source of antineoplastic molecules.

Table 5. Results of cytotoxicity evaluation.

| *Ficus sur* | Solvent | CC$_{50}$ ± SD (µg/mL) |
|-------------|---------|------------------------|
|             |         | VERO                   |
| Leaves      | MeOH    | >500                   |
|             | Infusion| >500                   |
| Stem bark   | MeOH    | 340.1 ± 22.72          |
|             | Infusion| 301.12 ± 30.31         |

Values are reported as mean ± SD of three parallel measurements.
however, to the best of our knowledge, this is the first report showing Ficus sur stem bark extracts as a possible source of antineoplastic molecules.

Figure 2. Influence of Ficus sur extracts on normal and cancer cells.

2.5. Antiviral Potential

The Ficus sur extracts were incubated with an HHV-1 infected VERO cell line to evaluate the antiviral potential. After CPE was found in the virus control cells, the influence on CPE was observed in extract-treated infected cells. It was found that only one extract, namely FLM at 250 µg/mL, decreased, but did not abolish altogether, CPE formation, as can be seen in Figure 3. The collected samples were further subjected to an end-point dilution assay to evaluate the infectious titer of HHV-1. The data on HHV-1 titer reduction contained in Table 6 confirmed that FLM 250 µg/mL exerted antiviral activity, decreasing the infectious titer by 2.86 log. However, since it is generally agreed that the tested sample should reduce the infectious titer by at least 3 log to show significant antiviral potential, FLM cannot be regarded as such. However, plant extracts are complex mixtures of compounds belonging to various groups of secondary metabolites, and the biological activity of such extracts depends on their composition, and the relative amount of particular substances and possible biological interactions (ex. synergism or antagonism). One of the end-point dilution assays performed for virus-infected cells treated is presented in Figure 4; in this particular experiment, the reduction of HHV-1 titer was 3.1 log. Considering this, the reported results can be regarded as interesting, and the observed antiviral activity will be further evaluated to elucidate the compounds responsible.
Table 6. Abatement of HHV-1 infectious titer in response to *Ficus sur* treatment.

| *Ficus sur* | Solvent | Concentration (µg/mL) | Decrease of HHV-1 Infectious Titer (Δlog *) |
|------------|---------|-----------------------|------------------------------------------|
| Leaves     | MeOH    | 250                   | 2.86 ± 0.17                              |
|            |         | 125                   | 1.03 ± 0.23                              |
|            | Infusion| 100                   | 0.52 ± 0.19                              |
|            |         | 50                    | 0.09 ± 0.16                              |
| Stem bark  | MeOH    | 125                   | 0.12 ± 0.06                              |
|            |         | 62.5                  | 0.07 ± 0.25                              |
|            | Infusion| 125                   | 0.92 ± 0.52                              |
|            |         | 62.5                  | 0.35 ± 0.13                              |

*Δlog (mean ± SD)-calculated from separate titration assays; Δlog = logCCID_{50}VC–logCCID_{50}E; VC–virus control; E–extract; Δlog ≥ 3 is regarded as significant.*

We have previously reported that *Oenanthe aquatica* and *Oenanthe silaifolia* extracts possess significant antiviral activity, and the observed effect may be related to the presence of caffeic acid and its derivatives (caffeic acid glucoside, chlorogenic acid, cryptochlorogenic acid, and neochlorogenic acid) present in those extracts [52]. Interestingly, caffeic acid derivatives were identified in the FLM, which showed the highest anti-HHV-1 activity, and in FSBI, which exerted a noticeable, though much lower, influence on the tested herpes virus, reducing the infectious titer only by 0.92 log. Furthermore, methyl gallate, detected exclusively in the FLM, was proven to be a potent and specific inhibitor of HHV-2 [53]. Additionally, FLM was the only extract showing the presence of 5,8-dihydroxy-7-methoxyflavone-O-glucoside-rhamnoside; there are reports of antiviral activity of some flavone compounds ex. 5,7-dihydroxy-3,4′-dimethoxyflavone (ermanin) and 5,7,4′-trihydroxy-3-methoxyflavone (isokaempferide) against polio [54] or 5,7,4′-trihydroxy-8-methoxyflavone against influenza virus [55], while 5-hydroxy-7-methoxyflavone and 5,7-dimethoxyflavone were found to be protease inhibitors active against HIV-1, HCV, and HCMV (Human cytomegalovirus, HHV-5, CMV) at micromolar concentrations [56]. The kaempferol-O-glucoside present in FLM was also isolated from *Securigera securidaca* and reported to inhibit HHV-1 attachment to the cell membrane, virus entry and viral polymerase [57], and showed potent anti-HIV-1 reverse transcriptase activity [58]. Flavone glycosides, namely quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside and kaempferol-3-O-robinobioside, were reported by Yarmolinsky et al. [59] as being responsible for the antiviral potential of *Ficus benjamina*. Interestingly, isolated glycosides exerted significant antiviral activity against HHV-1 and HHV-2, especially when added to infected cells during and after infection, but no activity was found against HHV-3 (varicella-zoster virus, VZV). Flavone aglycones, kaempferol and quercetin, obtained as standards, showed significantly lower activity [59]. Finally, FLM was the only extract that showed the presence of (epi)-afzelechin-7-O-glucoside, and of note, ent-epi-afzelechin-(4-8)-epiafzelechin was reported to inhibit HHV-2 by disrupting virus penetration and interfering with late stages of the viral replication cycle [60].
Figure 3. Influence of Ficus sur leaves methanolic extract on HHV-1 generated cytopathic effect.

Figure 4. Titration assay of HHV-1 in the sample treated with Ficus sur leaves methanolic extract.

3. Materials and Methods
3.1. Plant Materials and Preparation of Extracts

Ficus sur samples were collected in the village of Prikro (city of Brobo, Côte d’Ivoire), in January 2020. The species was identified by a plant taxonomist at the National Floristic Center (Université Felix Houphouet Boigny, Abidjan, Côte d’Ivoire). Voucher specimens were deposited at the herbarium of the above-mentioned center. The leaves and stem barks
of the plant samples were dried in shade conditions at room temperature for about one week. Then, the samples were powdered with a mill and stored in dark conditions.

Different solvents (methanol and water) were used to obtain the extracts in this study. Maceration was used as the extraction method for methanol extracts. In addition, the infusion was prepared. For the maceration, the plant materials (10 g) were macerated with 200 mL methanol at room temperature overnight. After that, the mixtures were filtered, and the solvents were evaporated. In preparing the water extracts, the plant materials (10 g) were kept with 200 mL boiled water for 15 min and then filtered. Water extracts were lyophilized, and all extracts were stored at 4 °C until analysis.

3.2. Chromatographic Conditions

The separation was performed on a C18 Gemini® column (3 µm i.d. with TMS end-capping, 110 Å, 100 × 2 mm) supported with a guard column (Phenomenex Inc, Torrance, CA, USA), at a flow rate of 0.2 mL/min under a gradient program operated by Agilent 1200 Infinity HPLC (Agilent Technologies, Santa Clara, CA, USA). Solvent A was water with 0.1% formic acid (v/v), whereas solvent B was 0.1% formic acid in acetonitrile (v/v). Both solvents were mixed according to the following program: 0–60% B for 45 min., next 60–95% B for 1 min., and 95% B for 4 min. The stop time was at 50 min. 10 µL of the sample was injected into a thermostated (20 °C) chromatographic column.

3.3. Detection Conditions

Mass spectra were acquired by the Agilent 6530B QTOF Accurate-Mass QTOF system equipped with Dual Agilent Jet Stream spray source (ESI) (Agilent Technologies, Santa Clara, CA, USA) connected with N2 generator (Parker Hannifin Corporation, Haverhill, MA; generating N2 at purities >99%). Negative ion mode was applied for MS and MS/MS acquisition with drying gas temp: 275 °C, drying gas flow: 10 L/min, sheath gas temp: 325 °C, sheath gas flow: 12 L/min; nebulizer pressure: 35 psig, capillary V (+): 4000 V, skimmer 65 V, fragmentor 140 V. Two spectra per sec were recorded in a range between 100 and 1000 m/z with a collision energy of 10 and 40 eV. The identification of compounds was based on fragmentation patterns and supported by a comparison of obtained mass spectra with those available in databases and the scientific literature.

3.4. Total Phenolic and Flavonoid Content

Total levels of phenolics and flavonoids were assessed based on previously reported methods [61,62]. Total phenolic levels were expressed as mg gallic acid equivalents (GAE)/g dry extract, and mg rutin equivalents (RE)/g dry extract was used to evaluate total flavonoids. All experimental details are given in the Supplementary Materials. The experiments were performed in triplicate, and the results were assessed by ANOVA assays (Tukey’s test).

3.5. Antioxidant and Enzyme Inhibitory Assays

In the current investigation, the antioxidant effects of the tested extracts were detected by different assays [61]. The assays were: [1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) radical scavenging, cupric ion reducing antioxidant capacity (CUPRAC), ferric ion reducing antioxidant power (FRAP), metal chelating ability (MCA) and phosphomolybdenum assay (PDA)]. For DPPH, ABTS, CUPRAC and FRAP assays, data were expressed as mg Trolox equivalents (TE)/g extract, whereas in MCA and PDA, mg EDTA equivalents (EDTAE)/g extract and mmol TE/g extract, respectively, were used. The experimental details for acetylcholinesterase, butyrylcholinesterase, tyrosinase, amylase and glucosidase assays were previously provided. Galanthamine was used as a positive control in cholinesterase assays, and data were evaluated as mg galanthamine equivalents (GALAE)/g extract. Kojic acid was used as a standard inhibitor in tyrosinase inhibitory assay, and the results were expressed as mg kojic acid equivalents (KAE)/g extract [61,62]. Acarbose was selected as an inhibitor
of both amylase and glucosidase in the antidiabetic assays, and the results are given as mmol acarbose equivalents (ACAE)/g extract. All experimental details are given in the Supplementary Materials. The assays were performed in triplicates, and the differences in the extracts were evaluated by ANOVA (Tukey’s test).

3.6. Cytotoxicity Testing

The evaluation of cytotoxicity was performed against normal kidney fibroblasts (VERO) and cancer cell lines derived from cervical adenocarcinoma (HeLa) and colon cancer (RKO) using microculture tetrazolium assay (MTT) as previously described [52]. Briefly, the cell monolayers were incubated with serial dilutions of the tested extracts for 72 h, and then cellular viability was assessed using the MTT protocol. Details can be found in the Supplementary Materials. The collected data were analyzed using GraphPad Prism to calculate the CC_{50} values (50% cytotoxic concentration). Additionally, selectivity indexes (SI) were calculated by comparing CC_{50} values obtained for VERO with those observed for cancer cells (SI = CC_{50,VERO}/CC_{50,Cancer}, SI > 1 indicates selectivity towards cancer cells).

3.7. Evaluation of Antiviral Potential

The extracts in non-toxic concentrations were tested for their influence on HHV-1 replication in the virus-infected VERO cells after 72 h incubation as previously described [52]. Briefly, the monolayer of VERO cells was treated with HHV-1 (100-fold CCID_{50}, CCID_{50}–50% cell culture infections dose) for 1 h, followed by washing with PBS (phosphate-buffered saline) and further incubated until a cytopathic effect (CPE) was recorded in the virus control (VC). Subsequently, after three cycles of freezing (−72 °C) and thawing, the HHV-1 infectious titer in the collected samples was measured using an end-point titration assay. Finally, the HHV-1 titer (Δlog) difference was calculated (Δlog = logCCID_{50,VC}–logCCID_{50,FE}, FE-Ficus extract). The difference of ≥3 log is regarded as significant.

4. Conclusions

In conclusion, the F. sur methanolic stem bark extract demonstrated substantial in vitro antioxidant potential with DPPH, ABTS, and CUPRAC assays, but not with FRAP, metal-chelating and phosphomolybdenum assays. The methanolic stem bark extract significantly depressed tyrosinase, α-amylase, AChE and BChE activity. To date, no evidence of enzyme inhibitory actions of Ficus members has been discovered. In this regard, the presented work is the first scientific demonstration of the enzyme inhibitory effects of F. sur extracts, and it may offer a substantial contribution to the scientific platform. Herein, we would like to report that the F. sur leaves methanolic extract exerted noticeable, but limited, antiviral activity against HHV-1, diminishing CPE development and reducing the virus titer by 2.86 log. Furthermore, antineoplastic activity against cervical adenocarcinoma and colon cancer cell lines was observed for stem bark infusion and methanolic extract. However, more study, including in vivo and clinical investigations, is needed to further examine these aforementioned properties to incorporate this traditional herb as a possible therapeutic element.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27061863/s1.

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References

1. Petrovska, B.B. Historical review of medicinal plants’ usage. *Pharmacogn. Rev.* 2012, 6, 1–5. [CrossRef] [PubMed]

2. Yoo, S.; Nam, H.; Lee, D. Phenotype-oriented network analysis for discovering pharmacological effects of natural compounds. *Sci. Rep.* 2018, 8, 11667. [CrossRef] [PubMed]

3. Stierle, A.; Strobel, G.; Stierle, D. Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. *Science* 1993, 260, 214–216. [CrossRef] [PubMed]

4. Murugesu, S.; Selamat, J.; Perumal, V. Phytochemistry, Pharmacological Properties, and Recent Applications of Ficus benghalensis and Ficus religiosa. *Plants* 2021, 10, 2749. [CrossRef] [PubMed]

5. Available online: http://www.worldfloraonline.org/ (accessed on 12 December 2021).

6. Clark, A.M. Natural products as a resource for new drugs. *Pharm Res.* 1996, 13, 1133–1144. [CrossRef] [PubMed]

7. Esievo, K.B.; Anthony, S.O.; Fatokun, O.T.; Kunle, O.F. Ficus capensis Thumb. (Moraceae): Review of its ethnomedicinal uses, pharmacological activities and phytochemical constituents. *Arch. Curr. Res. Int.* 2018, 12, 1–7. [CrossRef]

8. Lansky, E.P.; Paavilainen, H.M. *Figs: The Genus Ficus*; CRC Press: Boca Raton, FL, USA, 2010.

9. Sabiu, S.; O’Neill, F.H.; Ashafa, A.O.T. The Purview of Phytotherapy in the Management of Kidney Disorders: A Systematic Review on Nigeria and South Africa. *Afr J. Tradit. Complement. Altern. Med.* 2016, 13, 38–47. [PubMed]

10. Beyi, M. Ethnobotanical investigation of traditional medicinal plants in dugda district, Oromia Regio. *SM J. Med. Plant Stud.* 2018, 2, 1007.

11. Regassa, R. Diversity and conservation status of some economically valued indigenous medicinal plants in Hawassa College of Teacher Education Campus, Southern Ethiopia. *Int. J. Adv. Res.* 2013, 1, 308–328.

12. Ayele, M.; Makonnen, E.; Ayele, A.G.; Tolcha, Y. Evaluation of the Diuretic Activity of the Aqueous and 80% Methanol Extracts of Ficus sur Forsk (Moraceae) Leaves in Saline-loaded Rats. *J. Exp. Pharmacol.* 2020, 12, 619. [CrossRef] [PubMed]

13. Ishola, I.O.; Olayemi, S.O.; Yemitan, O.K.; Ekpemandudiri, N.K. Mechanisms of anticonvulsant and sedative actions of the ethanolic stem-bark extract of Ficus sur Forsk (Moraceae) in rodents. *Pak. J. Biol. Sci.* 2013, 16, 1287–1294. [CrossRef] [PubMed]

14. Diba, D.; Mekasha, Y.; Urge, M.; Tola, A. Feed intake, digestibility, growth performance, and blood profile of pigs fed mixtures of dried and ground fig (Ficus sur) fruits and graded levels of maize. *Trop. Anim. Health Prod.* 2015, 47, 339–346. [CrossRef] [PubMed]

15. Yarmolinsky, L.; Zaccai, M.; Ben-Shabat, S.; Mills, D.; Huleihel, M. Antiviral activity of ethanol extracts of Ficus benjamina and Lilium candidum in vitro. *New Biotechnol.* 2009, 26, 307–313. [CrossRef] [PubMed]

16. Camero, M.; Marinaro, M.; Lovero, A.; Elia, G.; Losurdo, M.; Buonavoglia, C.; Tempesta, M. In vitro antiviral activity of Ficus carica latex against caprine herpesvirus-1. *Nat. Prod. Res.* 2014, 28, 2031–2035. [CrossRef] [PubMed]

17. Lazreg Aref, H.; Gaaliche, B.; Fekih, A.; Mars, M.; Aouni, M.; Pierre Chaumon, J.; Said, K. In vitro cytotoxic and antiviral activities of Ficus carica latex extracts. *Nat. Prod. Res.* 2011, 25, 310–319. [CrossRef] [PubMed]

18. Huang, N.-C.; Hung, W.-T.; Tsai, W.-L.; Lai, F.-Y.; Lin, Y.-S.; Huang, M.-S.; Chen, J.-J.; Lin, W.-Y.; Weng, J.-R.; Chang, T.-H. Ficus septica plant extracts for treating Dengue virus in vitro. *PeerJ* 2017, 5, e3448. [CrossRef] [PubMed]

19. Cagno, V.; Civra, A.; Kumar, R.; Pradhan, S.; Donalisio, M.; Sinha, B.N.; Ghosh, M.; Lembo, D. Ficus religiosa L. bark extracts inhibit human rhinovirus and respiratory syncytial virus infection in vitro. *J. Ethnopharmacol.* 2015, 176, 252–257. [CrossRef] [PubMed]

20. Ghosh, M.; Civra, A.; Rittà, M.; Cagno, V.; Mavuduru, S.G.; Awasthi, P.; Lembo, D.; Donalisio, M. Ficus religiosa L. bark extracts inhibit infection by herpes simplex virus type 2 in vitro. *Arch. Virol.* 2016, 161, 3509–3514. [CrossRef] [PubMed]

21. Khairunisa, S.Q.; Indriati, D.W.; Tumewu, L.; Widyawaruyanti, A.; Nasronudin, N. Screening of anti-HIV activities in ethanol extract and fractions from Ficus fistulosa leaves. *J. Basic Clin. Physiol. Pharmacol.* 2021, 32, 734–742. [CrossRef]

22. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants* 2017, 6, 42. [CrossRef]

23. Zhang, Q.-W.; Lin, L.-G.; Ye, W.-C. Techniques for extraction and isolation of natural products: A comprehensive review. *Chin. Med.* 2018, 13, 20. [CrossRef] [PubMed]

24. Elhawary, S.S.; Younis, I.Y.; El Bishbishy, M.H.; Khattab, A.R. LC–MS/MS-Based chemometric analysis of phytochemical diversity in 13 Ficus spp. (Moraceae): Correlation to their in vitro antimicrobial and in silico quorum sensing inhibitory activities. *Ind. Crops Prod.* 2018, 126, 261–271. [CrossRef]

25. Farag, M.N.; Wu, W.; Kuhntert, N. The chlorogenic acids of Hemerocallis. *Food Chem.* 2006, 95, 574–578. [CrossRef]

26. Fotirić Aksić, M.; Dabić Zagorac, D.; Sredojević, M.; Milivojević, J.; Gašić, U.; Meland, M.; Natić, M. Chemometric characterization of strawberries and blueberries according to their phenolic profile: Combined effect of cultivar and cultivation system. *Molecules* 2019, 24, 4310. [CrossRef] [PubMed]
28. Gevrenova, R.; Zengin, G.; Sinan, K.I.; Yildiztugay, E.; Zheleva-Dimitrova, D.; Picot-Allain, C.; Mahomoodally, M.F.; Imran, M.; Dall’Acqua, S. UHPLC-MS Characterization and biological insights of different solvent extracts of two Achillea species (A. aleppica and A. santolinoides) from Turkey. Antioxidants 2021, 10, 1180. [CrossRef] [PubMed]

29. Guimarães, R.; Barros, L.; Dueñas, M.; Carvalho, A.M.; Queiroz, M.J.R.; Santos-Buelga, C.; Ferreira, I.C. Characterisation of phenolic compounds in wild fruits from Northeastern Portugal. Food Chem. 2013, 141, 5721–5730. [CrossRef] [PubMed]

30. Human Metabolome Database (HMDB). 2021. Available online: https://hmdb.ca/ (accessed on 31 July 2021).

31. MassBank of North America (MoNA) Database. 2021. Available online: https://mona.fiehnlab.ucdavis.edu/ (accessed on 31 July 2021).

32. Metlin Database. 2021. Available online: https://metlin.scripps.edu/ (accessed on 31 July 2021).

33. Puig-Seoane, A.; Cárdenas-Cuéllar, P.; Rodriguez-Cañizares, F.; López-Cabezudo, M.; Gómez-Gallego, J. Polyphenols and protein tyrosine phosphatase tyrosinase inhibition. J. Chromatogr. B 2013, 997, 58–65. [CrossRef] [PubMed]

34. Simirgiotis, M.J. Antioxidant capacity and HPLC-DAD-MS profiling of Chilean Peumo (Cryptocarya alba) fruits and comparison with German Peumo (Crataegus monogyna) from Southern Chile. Molecules 2013, 18, 2061–2080. [CrossRef]

35. Singh, A.; Kumar, S.; Kumar, B. LC-MS Identification of Proanthocyanidins in Bark and Fruit of six Terminalia species. J. Pharm. Biomed. Anal. 2020, 182, 1186935119842576. [CrossRef] [PubMed]

36. Swiatek, Ł.; Sieniawska, E.; Sinan, K.I.; Maciejewska-Turska, M.; Boguszewska, A.; Polz-Dacewicz, M.; Senkardes, I.; Guler, G.O.; Robin, V.; Boustie, J.; Amoros, M.; Girre, L. In-vitro antiviral activity of seven psiadia species, Asteraceae: Isolation of two antipoliovirus flavonoids from Psidia dentata. Pharm. Pharmacol. Commun. 1998, 4, 61–64.
55. Nagai, T.; Suzuki, Y.; Tomimori, T.; Yamada, H. Antiviral activity of plant flavonoid, 5, 7, 4′-trihydroxy-8-methoxyflavone, from the roots of Scutellaria baicalensis against influenza A (H3N2) and B viruses. *Biol. Pharm. Bull.* 1995, 18, 295–299. [CrossRef] [PubMed]

56. Sookkongwaree, K.; Geitmann, M.; Roengsumran, S.; Petsom, A.; Danielson, U.H. Inhibition of viral proteases by Zingiberaceae extracts and flavones isolated from Kaempferia parviﬂora. *Die Pharmazie—Int. J. Pharm. Sci.* 2006, 61, 717–721.

57. Behbahani, M.; Shanahezzadeh, M.; Shokoohinia, Y.; Soltani, M. Evaluation of anti-herpetic activity of methanol seed extract and fractions of Securigera securidaca in vitro. *J. Antivir. Antiretrovir.* 2013, 5, 72–76.

58. Behbahani, M.; Sayedipour, S.; Pourazar, A.; Shanahezzadeh, M. In vitro anti-HIV-1 activities of kaempferol and kaempferol-7-O-glucoside isolated from Securigera securidaca. *Res. Pharm. Sci.* 2014, 9, 463. [PubMed]

59. Yarmolinsky, L.; Huleihel, M.; Zaccai, M.; Ben-Shabat, S. Potent antiviral flavone glycosides from Ficus benjamina leaves. *Fitoterapia* 2012, 83, 362–367. [CrossRef] [PubMed]

60. Cheng, H.-Y.; Yang, C.-M.; Lin, T.-C.; Shieh, D.-E.; Lin, C.-C. ent-Epiafzelechin-(4α→8)-epiafzelechin extracted from Cassia javanica inhibits herpes simplex virus type 2 replication. *J. Med. Microbiol.* 2006, 55, 201–206. [CrossRef] [PubMed]

61. Grochowski, D.M.; Uysal, S.; Aktumsek, A.; Granica, S.; Zengin, G.; Ceylan, R.; Locatelli, M.; Tomczyk, M. In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of Potentilla thuringiaca. *Phytochem. Lett.* 2017, 20, 365–372. [CrossRef] [PubMed]

62. Uysal, S.; Zengin, G.; Locatelli, M.; Bahadori, M.B.; Mocan, A.; Bellagamba, G.; De Luca, E.; Mollica, A.; Aktumsek, A. Cytotoxic and enzyme inhibitory potential of two Potentilla species (*P. spectosa* L. and *P. reptans* Willd.) and their chemical composition. *Front. Pharmacol.* 2017, 8, 290. [CrossRef] [PubMed]