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(RESEARCH ARTICLE)

Phytochemical screening and antioxidant activity of methanolic extracts of 53 antimalarial plants from Bagira in Eastern DR Congo

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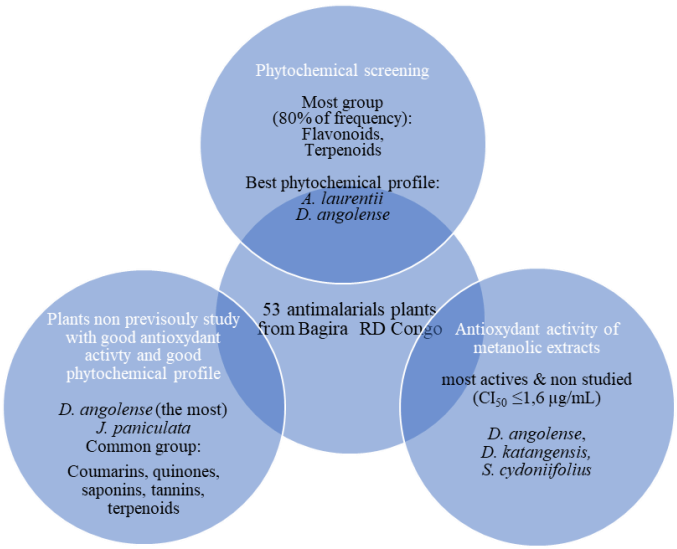
Abstract

A previous study inventoried 53 plants used in traditional medicine in Bagira in Eastern Democratic Republic of Congo (DRC) in the management of malaria. During malaria disease, oxidative stress is responsible for the worsening of the patient's condition. This study aims to identify phytochemical groups and to evaluate antioxidant activity of 53 plants used in traditional medicine in Bagira to treat malaria. The phytochemical screening was carried out by conventional reactions in solution and antioxidant activity used *in vitro* method with 1,1-diphenyl-2- picrylhydrazyl radical (DPPH). Chemical screening has identified secondary metabolites with both antimalarial and antioxidant potential such as coumarins, steroids, saponins, tannins and terpenoids in more than 70% of plants. Antioxidant screening revealed for the first-time antioxidant activity of 18 plants, among which *Dalbergia katangensis, Dialium angolense* and *Solanecio cydoniifolius* with $IC_{50} \leq 1.6$ µg / mL having the highest activities. This study shows that among plants used as antimalarial in Bagira several possess antioxidant power and contain many of groups presumed to be both antioxidant and antimalarial. This suggests that further studies continue to isolate compounds responsible for the proven activity.

Keywords: Anti-free radical activity; DPPH; Bagira; Antimalarial; Phytochemistry



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1. Introduction

Oxidative stress results from a profound imbalance between oxidative systems and the body's antioxidant capacities in favor of the former [1]. Unbalanced, it leads to irreversible cell damage [2] responsible for aging and many conditions such as obesity [3], type 2 diabetes [4], atherosclerosis [5], cancer [6] or virus diseases [7] requiring the use of antioxidants. Several synthetic antioxidants used in the past have been abandoned because of their increased risk of toxicity in favor of natural antioxidants [8], which motivates the screening of plants with antioxidant potential.

Studies have shown that during a malarial disease oxidative stress occurs which can progress to cerebral malaria or anemia [9,10]. Thus, studies have been carried out with a view to seeking both plants with antioxidant and antimalarial potential. This is the case with the work of Saliq et al [11] or Sulistyaningsih et al [12] like so many others [13–16]. Another advantage of this approach is that it allows the discovery of new antimalarial molecules with new mechanisms of action likely to overcome the resistance problems facing current antimalarials [17,18]. This approach to screening plants with dual potential has seen some studies lead to the isolation of natural molecules that are both antimalarial and antioxidant. This is the case of *mammea A / AA cyclo D*, a coumarin isolated from the stem bark of *Mesua borneensis* (P. F. Stevens), a Calophyllaceae [19] or that of *Lonchocarpol A*, a flavonoid isolated from the stem bark of *Erythrina crista-galli* L., a Fabaceae [20]. Furthermore, the isolation of a bioactive molecule is conventionally preceded by the search for secondary metabolites with the desired potential. Beyond this interest, this screening also makes it possible to provide new knowledge on the chemical composition of the plant concerned on the major phytochemical groups of secondary metabolites of plants. In the case of malaria, bibliographical reviews [21–23] have highlighted alkaloids, flavonoids, coumarins, quinones, steroids, terpenoids as phytochemical groups with antimalarial potential. Among these phytochemical groups, flavonoids, coumarins, and terpenoids are particularly reported as groups with antioxidant potential [24,25].

This study focused on 53 plants used in traditional medicine in Bagira, in the treatment of malaria, to assess their antioxidant potential in vitro and to search for phytochemical groups with antiplasmodial potential. These plants come from an ethnobotanical study carried out on antimalarial plants from Bagira, such as the city of Bukavu in the eastern DRC.

2. Material and methods

2.1. Plant material

The plant material consisted of the leaves, stems, roots, flowers, fruits, and aerial parts of 53 plant species taken from a database of a survey we conducted in Bagira in 2013-2014. These plants have been collected in Bukavu in the company of traditional healers and the herbaria created for this occasion were deposited at the IRS Lwiro herbarium where the identity of the plants was determined (Table 2). After drying at room temperature, the plant material was ground using a stainless-steel electric mill (Plymix PX-MFC 90 D, Belgium) and then kept cool before handling. The choice of organs to screen was related to availability at harvest. Thus, for the herbs, we screened the aerial parts consisting mainly of leaves and stems without discrimination.

2.2. Obtaining extracts

The extracts were obtained by maceration of 350 g of powder in 1.5 L of methanol (Sigma Aldrich, USA) for 72 hours at room temperature then filtered through paper (Whatman, USA) and concentrated on a rotary evaporator (Büchi R -210, Switzerland) at a pressure of 180 mbar and a temperature of 40 ° C.

2.3. Substrate and positive control

DPPH (Sigma Aldrich, United Kingdom) was used as a substrate for the evaluation of antioxidant activity. It was prepared at 0.002% (w / v) in methanol. L-ascorbic acid (Sigma Aldrich, China) used as a reference antioxidant substance made it possible to prepare a standard curve with 5 successive dilutions of order 2 carried out from a solution of ascorbic acid at 40 µg/mL ($y = 0.0298X + 0.0071$; $r^2 = 0.9997$).

2.4. Identification of secondary metabolites

The phytochemical screening was carried out using conventional reactions in solution in tubes, based on staining, precipitation, or the formation of foams. It consisted in looking for alkaloids, anthocyanins, coumarins, flavonoids, quinones, saponins, steroids, tannins and terpenoids for their antiplasmodial or antioxidant potential and cyanogenic heterosides for their toxic potential, following the protocols previously described [26–28].

2.4.1. Alkaloids

The detection of alkaloids consisted in precipitating them using six precipitation reagents. Briefly, 1 g of powder of dry plant material was macerated in 10 mL of methanol at room temperature for 24 hours and then in an oven at 50 ° C for 4 hours. The solution obtained was filtered then the marc washed three times with portions of hot methanol. The filtrate was evaporated to dryness in an oven at 50 ° C and the residue was collected twice with 2 mL of hot 1% hydrochloric acid solution (Sigma-Aldrich, USA). The acid solution obtained was basified with 1 mL of concentrated ammonia (Sigma-Aldrich, UK), placed in a separating funnel (VWR, Belgium) and then mixed with 5 mL of chloroform (Sigma-Aldrich, USA). After stirring, the two phases were separated, and the operation was repeated three times. The organic phase was evaporated to dryness in the open air, the residue obtained was taken up in 0.5 mL of chloroform and the solution, transferred to a test tube, was mixed with 0.5 mL of 1% HCl thus forming two phases. The aqueous phase, which is above, was removed using a Pasteur pipette. Six drops were placed on a microscope slide. Each of these drops was treated with one drop of one of six precipitation reagents namely Dragendorff, Mayer, Hager, Wagner, Bertrand, and Sonnenschein reagent. The presence of alkaloids was only considered certain if each of the six reagents gave a precipitate.

2.4.2. Coumarins

Coumarins were identified by the alkaline reaction. Briefly 0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for 5 minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

2.4.3. Flavonoids and anthocyanins

The flavonoids have been demonstrated by the Shinoda test. Briefly, 5 g of plant material placed in an Erlenmeyer flask was infused in 50 mL of distilled water for 30 minutes. 5 mL of filtrate were then treated successively with 5 mL of concentrated HCl, 5 drops of isoamyl alcohol and 1 mg of magnesium shavings. The red-orange (flavone), red or red-violet (flavonones), cherry red (flavonol) coloration appeared in the supernatant layer if the solution contained the flavonoids. Likewise, the reaction carried out for two minutes in a water bath in the absence of magnesium chips allowed the characterization of anthocyanins with the appearance of a red color.

2.4.4. Cyanogenic heterosides

Cyanogenic heterosides were identified by the reaction with picric acid. Briefly, 5 g of vegetable powder was placed in an Erlenmeyer flask with 10 mL of distilled water. The container was closed with a stopper to which was attached a strip of picrosodium paper lightly moistened with water and the contents were slightly heated (to 60 ° C). The yellow picrosodium paper turned orange or red if the plant extract had released hydrocyanic acid.

2.4.5. Quinones

Quinones were identified by the Borntrager test. Briefly, 5g of powdered plant material was macerated for 24 hours in 50mL of petroleum ether. After filtration, 10 mL of ethereal filtrate was treated with 5 mL of 10% NH_3. The appearance of a purplish red color in the aqueous phase indicated the presence of free quinones and that of yellow or orange colors, the bound quinones.

2.4.6. Saponins

Saponins were identified by the foaming reaction. Briefly, 10 g of coarsely ground plant material was treated with 100 mL of distilled water to make a decoction for 30 minutes and the mixture was filtered through filter paper after cooling. 15 mL of the decocts were then introduced into a test tube 16 mm in diameter and 160 mm in height. The contents of the tube were shaken tightly for one minute and then allowed to stand for 10 minutes. The appearance of a persistent foam greater than 10 mm in height indicates the presence of saponins.

2.4.7. Steroids and terpenoids

Both steroids and terpenoids have been defied by the reaction with sulfuric acid. Briefly, 5 g of plant material was macerated for 24 hours in 100mL of petroleum ether. After filtration, the solvent was evaporated to dryness. In the residue obtained, were added successively and with stirring, 2 mL of chloroform and three drops of concentrated sulfuric acid. The appearance of purple or green colorings indicated the presence of steroids. The identification of terpenoids followed the same pattern as that of steroids. In addition to the reagents used for steroid testing, a few drops

of Hirschson reagent (concentrated acetic anhydride) were added to 4 mL of the acidified solution. Yellow staining turning red indicated the presence of terpenoids.

2.4.8. Tannins

The tannins were identified according to the protocol below: 5 g of plant material were infused in 50 mL of water contained in an Erlenmeyer flask for 30 minutes. 5 mL of the infused was taken and mixed with 1 mL of 1% ferric chloride. The test was considered positive when either a precipitate appeared or a blue-green, dark blue or green color. 15 mL of Stiasny reagent (10 ml 40% formalin and 5 mL concentrated HCl) was mixed with 30 mL of the infused and the mixture was brought to a water bath at 90 ° C. The appearance of a precipitate indicated the presence of catechetical tannins. The solution was then filtered, and the filtrate was saturated with sodium acetate before adding a few drops of ferric chloride thereto. The formation of a precipitate in this case revealed the presence of gallic tannins.

2.5. Antioxidant activity test with DPPH

Antioxidant activity was assessed using the DPPH assay [29]. Briefly, 50 µL of extract or positive control prepared at different dilutions of order 2 in methanol from a 100 µg / mL solution were interacted with 1950 µL of 0.002% DPPH in test tubes. (Nunc WVR, Germany). After mixing and incubating in the dark for 30 minutes, the absorbance of the solution was read at 492 nm (Thermo Fisher Scientific Inc. spectrophotometer, Waltham, USA). The tests were carried out in triplicate and the percentage of antioxidant activity was calculated by the formula:

$$\%AAO = \frac{(Ab-Ae)x100}{Ab} \text{ (equation 1)}$$

with Ab = absorbance measured in the presence of the negative control, Ae = absorbance measured in the presence of the extract and % AAO = Percent inhibition and expresses antioxidant activity. This percentage of activity made it possible to generate the IC_{50} or concentration at which the extract has 50%, to categorize the extracts.

2.6. Statistical analysis of data

GraphPad Prisme version 6 software (GraphPad Software, La Jolla, USA) was used to perform statistical analysis of the data and generate the IC_{50}s. The analysis of the variables was carried out by one-way ANOVA with the significance level set at 95%.

3. Results and discussion

3.1. Phytochemical screening

The results of the chemical screening show that each of the 53 plants contains at least 5 phytochemical groups out of the 10 sought. No plant contains cyanogenic heterosides and 3 species, *Carica papaya*, *Entada abyssinica* and *Flueggea virosa*, all contain the 9 groups with therapeutic potential. Each organ contains at least 4 phytochemical groups and the leaves of *Azadirachta indica* as well as the fruits of *Lantana camara* with 8 groups each, contain the greatest number of the desired phytochemical groups (Table 1).

Table 1 shows that antimalarial molecules have already been isolated for 12 plants. These are the species *Artemisia annua* (terpenoids), *Azadirachta indica* (terpenoids), *Bidens pilosa* (flavonoids), *Cajanus cajan* (flavonoids), *Cymbopogon citratus* (terpenoids), *Erythrina abyssinica* (flavonoids), *Euphorbia hirta* (flavonoids), *Lantana camara* (terpenoids), *Ochna schweinfurthiana* (flavonoids), *Phyllanthus niruri* (steroids), *Physalis angulata* (steroids) and *Tithonia diversifolia* (terpenoids). It also shows that 11 plants were so far phytochemically unrecognized. These are, *Aframomum laurentii, Clematis villosa*, *Crassocephalum montuosum*, *Crassocephalum picridifolium*, *Dalbergia katangensis*, *Dialium angolense*, *Isoberlinia angolensis*, *Isoberlinia tomentosa, Julbernardia paniculata*, *Rothmannia engleriana*, and *Solanecio cydoniifolius*.

Table 1 Phytochemical screening of 53 plants used as antimalarial drugs in Bagira (DRC)

	Species	PU	Alkaloids	Anthocyanins	Coumarins	Flavonoids	Quinones	Saponins	Steroids	Tannins	Terpenoids	Hcn	Previous chemical screening
1	*Acacia polyacantha* De Wild (Fabaceae)	F	+[a]	+[a]	-	-	-[a]	+[a]	-	+	-	-	[30]
		ET	+	-[a]	+	-	+	+[a]	-	+	+	-	
		R	+[a]	-[a]	-	+	-	+[a]	-	+	+	-	
		Fr	-	+	-	+	+	+	-	+	+	-	
		Flr	-	+	-	+	-	+	-	+[a]	+	-	
2	*Aframomum laurentii* (De Wild & T, Durand) K. Schum (Zingiberaceae)	PA	-	-	-	+	++	-	-	-	++	-	
		F	-	-	+	+	+		+	-	-	-	
		R	-	+	-	-	+	+	-	+	+	-	
		Fr	-	+	-	+	+	+	+	+	+	-	
3	*Ageratum conyzoïdes* L. (Asteraceae)	F	+[a]	+	-[a]	+[a]	-	++	+[a]	+	+[a]	-	[31]
		ET	+[a]	+	-	+	-	+	+	(+)	+	-	
		ER	+	-	-	-	-	+	-	+	+	-	
4	*Artemisia annua* L. (Asteraceae)	F	-	-	+	+[a]	-	-	+a	-	++[b]	-	[32,33]
		R	-	-	-	+	+	+	+	-	++	-	
5	*Azadirachta indica* A. Juss (Meliaceae)	F	+a	-	+	+a	+	+	+a	+	+b	-	[34,35]
		ET	+	-	-	+	-	+	+	+	-	-	
		ER	+	-	-	+	-	-	+	+	+b	-	
6	*Bidens pilosa* L. (Asteraceae)	F	-[a]	-	-	+[b]	-	+[a]	+[a]	+[a]	-[a]	-	[36,37]
		R	-	+	-	+	+	+	-	-	-	-	
7	*Bobgunia madagascariensis* (Desv.) J.H. Kirkbr. (Fabaceae)	F	-	++[a]	-	+[a]	-	+	+	+	-	-	[38]
		ET	-	-	-	-	++[a]	+	+	+[a]	-[a]	-	
		ER	-	+	-	-	-	+	-	+	+	-	
8	*Cajanus cajan* (L.) Millsp. (Fabaceae)	F	-[a]	-	-	+[b]	+	+[a]	+	+[a]	++[a]	-	[39,40]
		ET	-	-	-	+	+	+	+	+	+	-	
		R	-	-	-	+	(+)	-	(+)	(+)	+	-	
		Fr	-	+	-	+	-	+	+	+	-	-	
9	*Carica papaya* L(Caricaceae)	F	+[a]	-	-	+[a]	+	-	+[a]	-	+	-	[41]
		ET	-	-	-	+[a]	+	-	+[a]	+	-	-	
		ER	-	-	-	+a	+	-	+[a]	+	+	-	
		Fr	-	+	+	+[a]	+	-	+[a]	+	+	-	
		Flr	-	++	-	+	-	+	+	+	+	-	
10	*Cassia occidentalis* L. (Fabaceae)	F	+[a]	-	-	+[a]	-	+	-	-	+[a]	-	[42]
		T	+	-	-	+[a]	-	+	-	+[a]	-	-	
		R	+[a]	-	-	+[a]	-	+	-	+	-	-	
		Fr	+	+	++	+[a]	-	+	-	-	+	-	
		Flr	+	++	-	+	-	+	-	-	+	-	
11	*Catharanthus roseus* (L.) G Don. (Apocynaceae)	F	+[a]	-	-	+[a]	-	-	+	+[a]	-	-	[43]
		T	+	-	+	-	+[a]	+	-	-	+	-	
		R	+	-	-	+	+	-	+	-	-	-	
		Flr	+	++	-	+	-	+	+	+	-	-	

12	*Chenopodium abrosioides* L. (Chenopodiaceae)	F	+[a]	-	++[a]	+[a]	-	-	+	-	+[a]	-	[44]
		R	-	-	++[a]	+	-	+[a]	-	-	+	-	
13	*Chenopodium opulifolium* Schrad, EX Wdj. Koch (Chenopodiaceae*)*	PA	-	+	-	+[a]	+	++	+	+	+[a]	-	[45]
14	*Cinchona ledgeriana* (Howard) Bern. Moens Ex Trimen (Rubiaceae)	F	+[a]	-	+[a]	+	+[a]	-	+	-[a]	-	-	[46]
		ET	++[a]	-	+[a]	+	+[a]	+	+	-[a]	-	-	
		ER	+[a]	-	+[a]	-	+[a]	-	+	-	-	-	
		Flr	-	++	-	++	-	+	+	-	+	-	
15	*Clematis villosa* DC (Ranunculaceae)	PA	+	-	+	-	++	-	++	-	+	-	
16	*Crassocephalum montuosum* (S. Moore) Milne-Redh (Asteraceae)	PA	-	+	+	-	-	+	-	+	+	-	
17	*Crassocephalum picridifolium* (DC) S More (Asteraceae)	PA	-	-	+	+	-	+	+	-	+	-	
18	*Cymbopogon citratus* (DC) Stapf. (Poaceae)	F	-	+	-	+	+	-	-	-	+[b]	-	[47,48]
		R	-	-	-	++	+	-	+[a]	-[a]	+	-	
19	*Dalbergia katangensis* Lechenaud (Fabaceae)	F	-	-	+	+	++	++	-	-	-	-	
		ET	-	-	+	-	+	+	-	+	++	-	
20	*Dialium angolense* (Welw EX Beth) Harms (Fabaceae)	F	-	++	-	+	+	-	+	+	-	-	
		ET	-	-	(+)	-	+	+	+	+	+	-	
		ER	-	+	+	-	-	+	-	+	+	-	
21	*Dialopsis africana* Radck (Sapindaceae)	F	-	+	-	++	++	+	-	-	+	-	[49]
		ET	-	-	+	+	+	+[a]	+	+	-	-	
		ER	-	+	-	-	-	+	+	+	-	-	
22	*Ekebergia benguellensis* Welw EX CDC (Meliaceae)	F	-	++[a]	++	+[a]	+	+	++	++[a]	++[a]	-	[50,51]
		ET	-	+	-	-	-	+	-	+	+	-	
		ER	-	++	++	-	-	+	++	-	+	-	
23	*Eleusine indica* (L) Gaertn (Poaceae)	PA	-[a]	+	-	+[a]	-	+		+[a]	+	-	[52,53]
24	*Entada abyssinica* Steud. ex A. Rich. (Fabaceae)	F	+[a]	-	-	+	-	-	+	+	-	-	[30]
		ET	-	+	-	+[a]		+[a]	-[a]	+[a]	-	-	
		R	+	-	-	+[a]	+	+[a]	+[a]	+a	+	-	
		Fr	-	++a	+	+	+	+		+	+	-	
25	*Erythrina abyssinica* Lam. Ex DC (Fabaceae)	F	++	+	-	+[b]	++	+	++	-	+	-	[54,55]
		ET	++	+	-	+[a]	+	+	+	-[a]	++	-	
		R	+++[a]	+	-	+[a]	+	+	+	-[a]	+++	-	
26	*Euphorbia hirta* L. (Euphorbiaceae)	F	-	-	+	++[b]	-	+	-	+	+	-	[56,57]
		PA	+[a]	+	+	+[a]	-[a]	-	+	+[a]	+[a]	-	
27	*Flueggea virosa* (Roxb. Ex Willd.) Voigt (Phyllanthaceae)	F	-	+	-	+	+	-	+[a]	-	-	-	[58]
		R	+[a]	-	-	+	+[a]	-	-	+	-	-	
		Fr	-	+	-	+	-	+a	-	-	+	-	
28	*Hypoestes triflora* (Forssk) Roem, & Schult (Acanthaceae)	PA	+	+	+	-	++	-	+[a]	-	-	-	[59,60]

29	*Isoberlinia angolensis* (Welw. Ex Benth.) Hoyle & Brenan (Fabaceae)	PA	+	-	+	-	+	+	-	+	-	-	
30	*Isoberlinia tomentosa* (Harms) Craib & Stapf (Fabaceae)	PA	-	+	+	+	-	-	+	-	+	-	
31	*Jatropha curcas* L. (Euphorbiaceae)	F	+[a]	-	-	+[a]	-	-[a]	+	-[a]	+	-	[61]
		T	-	-	-	+	+	+	-	-	+	-	
		R	+	-	-	+	-	-	+	+	-	-	
		Fr	-	+	-	+	-	-	+	-	+	-	
32	*Julbernardia paniculata* (Benth.) Troupin (Fabaceae)	F	-	+	-	-	-	+	++	+	-	-	
		ET	-	+	-	-	+	+	++	+	-	-	
		ER	-	-	+	-	+	-	++	+	+	-	
33	*Lantana camara* L (Verbenaceae)	F	+[a]	+	-	+[a]	-	-[a]	-[a]	-	++[b]	-	[62]
		T	-	-	-	+	-	+	-	+	++	-	
		R	-	-	-	+	-	+	+[a]	-	++	-	
		Fr	+	+	-	+[a]	+[a]	+	+[a]	+	++	-	
		Flr	+	-	-	+[a]	+[a]	-	+[a]	+	++	-	
34	*Leucas martinicensis* (Jacq.) R. BR. (Lamiaceae)	F	-[a]	-	+	+[a]	-[a]	-	+[a]	-[a]	+	-	[63]
		R	+	-	-	+	+	-	-	+	-	-	
35	*Mangifera indica* L. (Anacardiaceae)	F	-[a]	-	-	+[a]	-	-[a]	+	+[a]	+	-	[64]
		ET	+[a]	-	-	+[a]	-	+	-	-	+	-	
		ER	-	-	-	+	-	+	-	+	+	-	
		Fr	-	+	-	+	-	-	+	+	+	-	
36	*Moringa oleifera* Lam. (Moringaceae)	F	+[a]	-	-	+[a]	-[a]	+	-	+[a]	+[a]	-	[65,66]
		T	-	-	+	+	-	-	+	-	+	-	
		R	+	-	-	+	-	+[a]	-	+[a]	+[a]	-	
		Fr	-	+	-	+	-	-	+	-	+	-	
37	*Ochna schweinfurthiana* F Hoffm (Ochnaceae)	F	-[a]	++	+	++[b]	++	+[a]	-[a]	+[a]	+[a]	-	[67]
		ER	-	+	(+)	-	-	-	++	-	+	-	
		ET	-	+	-	-	-	+	+	+	-	-	
38	*Ocimum gratissimum* L. (Lamiaceae)	F	-[a]	+	-	+[a]	-	+[a]	+	-[a]	+[a]	-	[68,69]
		R	-	+	-	+	-	+	+	-	+	-	
		Flr	-	+	-	+	-	-	+	-	+	-	
39	*Phyllanthus muellerianus* (Kuntze) Exell (Phyllanthaceae)	F	-[a]	-	+	+[a]	-	+[a]	+[a]	+[a]	+[a]	-	[70]
		ET	-	-	-	+	-	+	+	+	+	-	
		R	-	-	+	+	-	+	+	+	+	-	
		Fr	-	+	+	+	-	+	+	+	+	-	
40	*Phyllanthus niruri* L. (Phyllanthaceae)	F	+	-	-	+	-	+	+[b]	-	+	-	[71]
		T	-	-	-	+	+	-	+	-	+	-	
		R	+[a]	-	-[a]	+[a]	-	+[a]	-	-[a]	+[a]	-	
41	*Physalis angulata* L. (Solanaceae)	F	-	+	-	+	+	+	+	-[b]	-	-	[72,73]
		R	+[a]	-	-	+[a]	-	-	+[a]	-	++	-	
		Fr	-	+	-	+	+	-	+	-	+	-	
42	*Piliostigma thonningii* (Schum.) Milne-Redh. (Fabaceae)	F	-	+	-	+	-	+	+	+	+	-	[74]
		T	-[a]	+	+	-[a]	-	+[a]	-	-[a]	+[a]	-	
		R	-	-	+	+	+[a]	+	+	-	+	-	

		Fr	-	+	-	+	+[a]	-	+	+	-	-	
43	*Psidium guajava* L. (Myrtaceae)	F	-	+	-	+[a]	-	+[a]	+[a]	-[a]	+[a]	-	[75]
		ET	-	-	+	+[a]	+	-[a]	+[a]	-[a]	+[a]	-	
		ER	-	+	-	+[a]	-	-[a]	+[a]	+[a]	-[a]	-	
		Fr	-	+++	-	+[a]	-	-[a]	+[a]	-[a]	+[a]	-	
44	*Psorospermum corymbiferum* Spach (Hypericaceae)	F	-	+	-	+	-	++	++	++	-	-	[76]
		ER	-	-	+	++	+[a]	-	++[a]	+[a]	++[a]	-	
		ET	-[a]	+	+	+	+	+	+	-	+	-	
45	*Rothmannia engleriana* (K. Shum) Keay (Rhubiaceae)	F	-	+	++	-	++	++	++	+	+	-	
		ER	-	+	++	-	-	++	++	-	++	-	
		ET	-	+	-	-	+	-	-	+	+	-	
46	*Senecio cineraria* (DC) (Asteraceae)	PA	+[a]	+	-	+[a]	++	+	+	-[a]	+	-	[77]
47	*Solanecio cydoniifolius* (O Hoffm.) C. Jeffrey (Asteraceae)	PA	-	+	+	+	-	-	-	+	+	-	
48	*Spilanthes mauritiana* (A. Rich. Ex Pers.) DC. (Asteraceae)	F	-[a]	-	-	+[a]	+[a]	+	+	-	-	-	[78]
		Flr	-	+	-	+	-	-	+	-	+	-	
49	*Syzygium cordatum* Hochst. in C. Krauss (Myrtaceae)	F	-	+[a]	-	+[a]	+	+	-	-	-	-	[79]
		ET	-	+[a]	-	+[a]	-	+	-	+[a]	+[a]	-	
		R	-	+[a]	-	+[a]	-	-	+	+	-	-	
		Fr	-	+	-	+	-	+	-	-	+	-	
50	*Tagetes minuta* L. (Asteraceae)	F	+[a]	-	-	+[a]	-	-	+[a]	-	+[a]	-	[80]
		R	+	-	-	+	-	+[a]	+[a]	-	-	-	
		Flr	+	++	-	+	-	-	+	+	+	-	
51	*Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae)	F	+	-	-	+a	-	++	+	-	+++[b]	-	[81]
		T	+	+	-	+a	-	++	(+)	(+) [a]	++[a]	-	
		R	-	+	-	+	-	++	-	(+)	-	-	
		Flr	+	+	-	+	-	+	-	+	-	-	
52	*Trema orientalis* (L.) Blume (Ulmaceae)	F	-	+	-	+[a]	+	+[a]	+	-[a]	+[a]	-	[82]
		T	-	-	+	+	+	-	+	-	-	-	
		R	-	-	+	+	-	+	+	-	+	-	
53	*Vernonia amygdalina* Delille (Asteraceae)	F	+[a]	+	-	+[a]	-	-	-	-	+	-	[83]
		T	-	+	-	+	+[a]	+[a]	-[a]	+[a]	+	-	
		R	+	+	-	+	+	+	-	+	+	-	

PU: Part used; Hcn: cyanogenic heterosides; +: presence; -: Absence; (+):presence in trace; a group already identified during previous work; b phytochemical groups within which antimalarial molecules have been previously identified; F: leaves; R: roots; T: rod; ET: stem bark; ER: root bark; Flr: Flowers; PA: aerial parts.

3.1.1. Classification of species according to the number of phytochemical groups identified

Depending on the number of phytochemical groups identified within each plant, all the organs together, the 53 plant species can be grouped into 5 classes (Classes A to E). Although almost 70% of plant species contain 7 phytochemical groups, only 6% of plants contain the nine phytochemical groups with therapeutic potential (Figure 1).

Class A: species with 5 phytochemical groups; Class B: species with 6 phytochemical groups; Class C: species with 7 phytochemical groups; Class D: species with 8 phytochemical groups; class E: species with 9 phytochemical groups.

The class A includes 10 species which are *Clematis villosa, Crassocephalum montuosum, Crassocephalum picridifolium, Cymbopogon citratus, Eleusine indica, Hypoestes triflora, Isoberlinia angolensis, Isoberlinia tomentosa, Ocimum gratissimum* and *Solanecio cydoniifolius*. Class B includes 7 species: *Artemisia annua, Bidens pilosa, Chenopodium abrosioides, Chenopodium opulifolium, Dalbergia katangensis, Phyllanthus niruri, and Spilanthes mauritiana*. We find in class C, 17 species, *Ageratum conyzoïdes, Bobgunia madagascariensis, Cajanus* cajan, *Cassia occidentalis, Erythrina abyssinica, Julbernardia paniculata, Leucas martinicensis, Mangifera indica, Physalis angulata, Rothmannia engleriana, Senecio* cineraria, *Syzygium cordatum, Tagetes minuta, Tithonia diversifolia, Trema orientalis*, and *Vernonia amygdalina.* Class D has only 3 species, these are *Carica papaya*, *Entada abyssinica and Flueggea* virosa. In class E we find the species *Acacia polyacantha*, *Aframomum laurentii*, *Azadirachta indica*, *Catharanthus roseus*, *Cinchona ledgeriana, Dialium angolense, Dialopsis africana, Ekebergia benguellensis*, *Euphorbia hirta, Jatropha curcas*, *Lantana camara, Mangifera indica, Ochna schweinfurthiana, Piliostigma thonningii, Psidium guajava*, and *Psorospermum corymbiferum*.

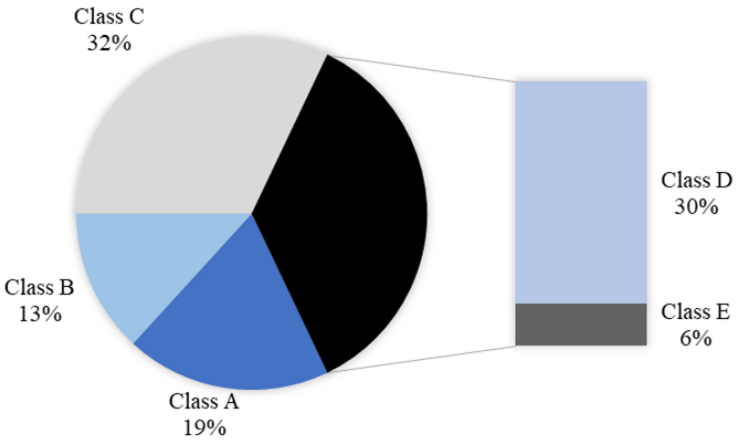

Figure 1 Classification of species according to the number of phytochemicals identified within the species, N = 53.

3.1.2. Classification of phytochemical groups identified in the 53 plants

The phytochemical groups identified can be classified either according to the overall result of the screening or according to the result within each organ. According to the overall results of the phytochemical screening, flavonoids (81.7%) and terpenoids (70.5%) are the most representative, while quinones (29.8%) and alkaloids (35.8%) are the most representative less frequent (figure 2).

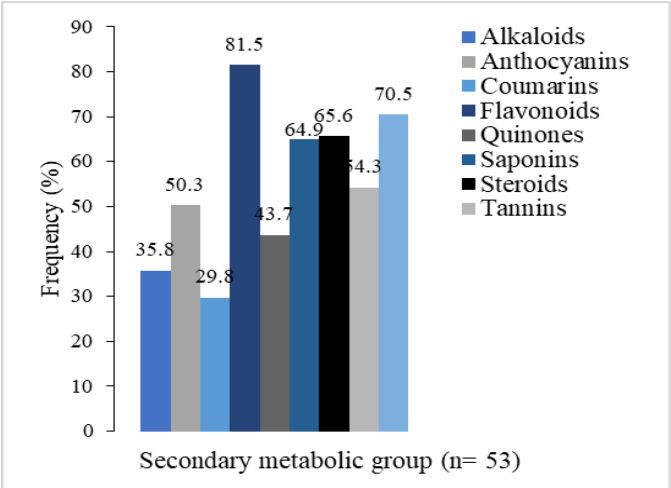

Figure 2 Frequency of phytochemical groups from 53 plants.

These frequencies observed for the entire screening (Figure 2) are not, however, in the same proportions for each organ (Figure 3). Flavonoids, for example, which are 81.7% overall, vary between 67 and 100% depending on the organ (Figure 3), thus illustrating a variability in the chemical composition of species. This variability in the composition of

secondary metabolites within the same plant may justify the difference in pharmacological properties attributable to each species depending on the organ considered.

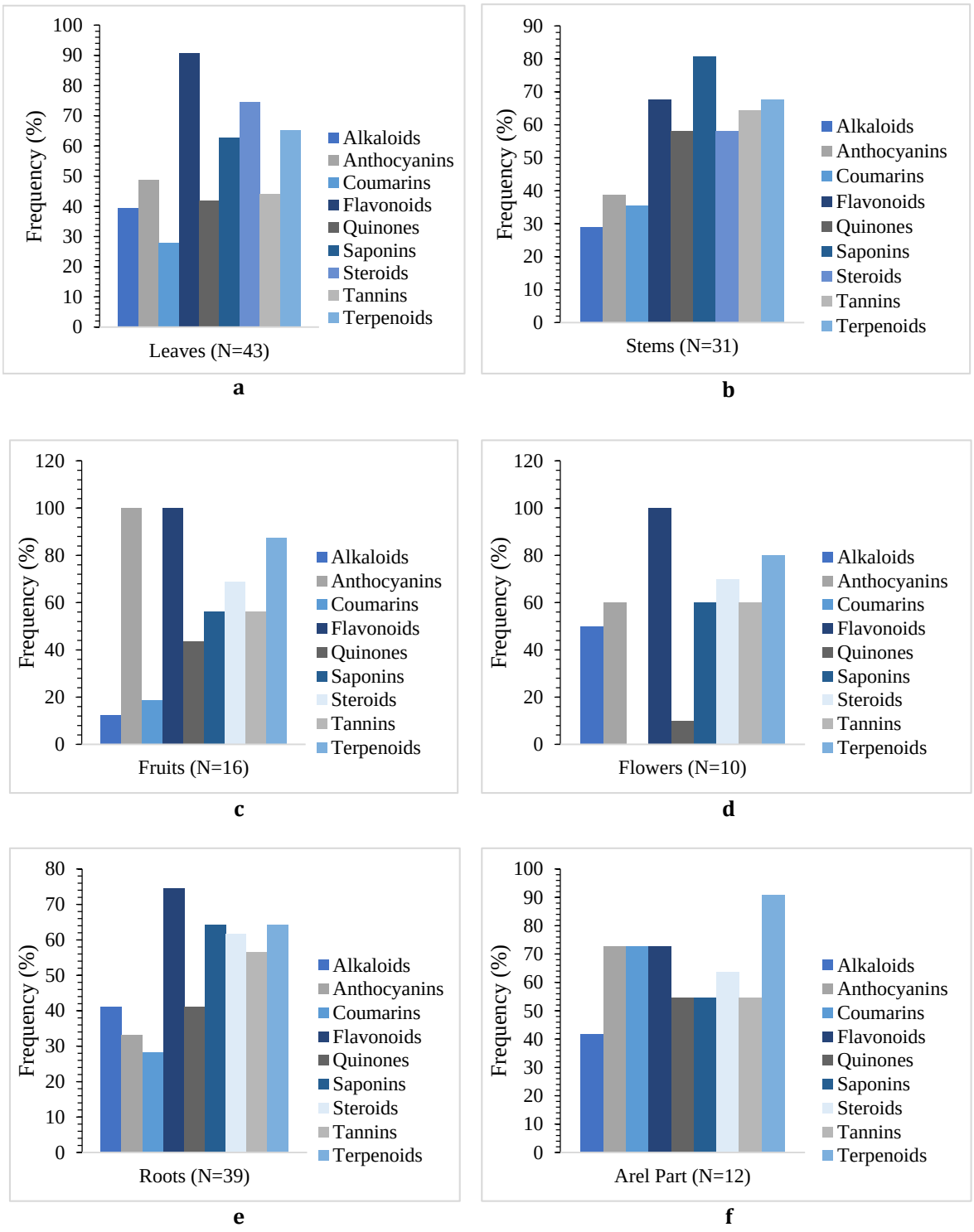

Figure 3 Frequency of secondary metabolites in different organs of plants: leaves (a), stems (b), fruits (c), flowers (d), roots (e) and aerial parts (f).

3.2. Antioxidant activity of methanolic extracts from 53 selected plants

Regarding the IC_{50} values, the 147 extracts obtained from the 53 plants can be grouped into 4 classes. Class 1 is that of very active extracts ($IC_{50} \leq 1.6$ µg / mL), class 2 is that of active extracts ($1.6 < IC_{50} \leq 50$ µg / mL), class 3 contains weakly

active extracts (50 <IC_{50} ≤ 200 µg / mL) and class 4 contains inactive extracts (IC_{50}> 200 µg / mL). In addition, 68% of plants have already been evaluated for antioxidant activity (Table 2).

Table 2 Antioxidant activity of methanolic extracts from 53 plants known to be antimalarial in Bagira.

			CI_{50}: Mean ± SD (µg/mL) N=3				
	Ascorbic acid		**1.01 ± 0.4**				
	Species	**Herbarium Code**	**Leaves**	**Root**	**Stem**	**Areal part**	**Organ: Previous antioxidant activity**
1	*Acacia polyacantha*	KH5128	48.4 ± 1.4[b]	38.8 ± 0.4[b]	60.4 ± 1.1[d]		G : [84]
2	*Aframomum laurentii*	KH4072	>200	45.1±0.2[b]		>200	
3	*Ageratum conyzoïdes*	KH3560	25.5 ± 0.1[b]	18.5 ± 0.2 [a]	75.5 ± 0.4[d]		PA : [85]
4	*Artemisia annua*	IL2045	>200	>200			F, PE : [86]
5	*Azadirachta indica*	IL2451	13.2 ± 0.4[a]	8.1 ± 1.5[a]	75.5 ± 1.7[d]		T : [87] F : [35]
6	*Bidens pilosa*	IL8431	1.1 ± 0.4	1.2 ± 0.1			F: [88][79]
7	*Bobgunia madagascariensis*	IL4757	30.9 ± 0.4[b]	11.1 ± 0.4[a]	13.1 ± 0.4[a]		
8	*Cajanus cajan*	IL6158	30.9 ± 2.4[b]	10.1 ± 0.4[a]	13.4 ± 0.4[a]		F : [89]
9	*Carica papaya*	IL3671	13.1 ± 1.5[a]	13.3 ± 1.4[a]	14.1 ± 0.4[a]		F : [90]
10	*Cassia occidentalis*	IL3978	13.1 ± 0.4[a]	13.1 ± 0.4[a]	63.9 ± 1.6[d]		PA, F : [91]
11	*Catharanthus roseus*	IL4292	30.8 ± 1.4[b]	>200	7.4 ± 1.4[a]		R : [92][91] F : [93]
12	*Chenopodium abrosioides*	IL8462	13.1 ± 1.1[b]	>200			[94]
13	*Chenopodium opulifolium*	IL4012	12.1 ± 0.2[b]			>200	
14	*Cinchona ledgeriana*	IL3076	30.8 ± 0.2[b]	13.1 ± 0.2[a]	>200		F :[46]
15	*Clematis villosa*	IL3076				47.7 ± 0.2[c]	
16	*Crassocephalum montuosum*	IL1546				60.2 ± 0.2[d]	
17	*Crassocephalum picridifolium*	IL1498				47.7 ± 0.2[c]	
18	*Cymbopogon citratus*	Il2098	59.9 ± 0.2[c]	13.1 ± 0.2[a]			R, PA : [95] F : [96]
19	*Dalbergia katangensis*	IL1025	1.1 ± 0.1				
20	*Dialium angolense*	IL1087	1.2 ± 0.2	7.4 ± 0.2[a]			
21	*Dialopsis africana*	IL1091	1.4 ± 0.1	7.4 ± 0.2[a]	>200		
22	*Ekebergia benguellensis*	IL2076	1.2 ± 0.3	>200	>200		
23	*Eleusine indica*	IL3061				13.1 ± 0.2[a]	PA : [97]
24	*Entada abyssinica*	IL1546	30.7 ± 0.2[b]	13.1 ± 0.2[a]	>200		F : [98]
25	*Erythrina abyssinica*	IL1498	7.4 ± 0.2[a]	13.1 ± 0.2[a]	>200		F, T, R : [99]
26	*Euphorbia hirta*	I L2098	51.8 ± 0.2[c]				PA : [100]

27	*Flueggea virosa*	IL1025	>200	>200			
28	*Hypoestes triflora*	IL1087				58.6 ± 0.2^{c}	
29	*Isoberlinia angolensis*	IL1091				59.6 ± 0.2^{c}	
30	*Isoberlinia tomentosa*	IL2076				13.1 ± 0.1^{c}	
31	*Jatropha curcas*	IL3061	>200	7.4 ± 0.2^{a}	>200		F: [101]
32	*Julbernardia paniculata*	IL1098	>200	13.1 ± 0.2^{a}			
33	*Lantana camara*	IL1075	50.2 ± 0.2^{c}	>200	58.9 ± 0.2^{c}		F, T, R, Flr :[102]
34	*Leucas martinicensis*	IL713	7.4 ± 0.2^{a}	>200			PE : [103]
35	*Mangifera indica*	IL3026	13.1 ± 0.2^{b}				F : [104] Fr :[105]
36	*Moringa oleifera*	IL1233	30.7 ± 0.2^{b}	7.4 ± 0.2^{a}	>200		F :[14]
37	*Ochna schweinfurthiana*	IL1063	7.4 ± 0.2^{a}	>200	13.1 ± 0.2^{a}		
38	*Ocimum gratissimum*	IL1087	30.7 ± 0.2^{b}	>200			F, T : [106]
39	*Phyllanthus muellerianus*	IL4679	30.7± 0.2^{b}	13.1 ± 0.2^{a}	>200		PA: [107]
40	*Phyllanthus niruri*	IL1076	30.9 ± 0.2^{b}	7.4 ± 0.2^{a}	>200		F & Fr: [108]
41	*Physalis angulata*	IL4078	13.1 ± 0.2^{a}	13.1 ± 0.2^{a}	>200		[109]
42	*Piliostigma thonningii*	IL2045	30.9 ± 0.2^{b}	>200	>200		F: [110]
43	*Psidium guajava*	IL0241	30.9 ± 0.2^{b}	13.1 ± 0.2^{a}	13.1 ± 0.2^{a}		F : [111]
44	*Psorospermum corymbiferum*	IL2089	>200	7.4 ± 0.2^{a}	10.1 ± 0.2^{a}		F : [76]
45	*Rothmannia engleriana*	IL3075	7.4 ± 0.2^{a}	13.1 ± 0.2^{a}	>200		
46	*Senecio cineraria*	IL1065				1.5 ± 0.3	PE :[77]
47	*Solanecio cydoniifolius*	IL3070				1.6 ± 0.1	
48	*Spilanthes mauritiana*	IL7012	1.6 ± 0.3^{a}				PE: [78]
49	*Syzygium cordatum*	IL4051	14.1 ± 0.2^{a}	7.4 ± 0.2^{a}	>200		Fr :[112]
50	*Tagetes minuta*	IL2458	12.1 ± 0.2^{a}	>200			PA : [113]
51	*Tithonia diversifolia*	IL2097	30.7 ± 0.2^{b}	>200	7.4 ± 0.2^{a}		F :[102]
52	*Trema orientalis*	IL7849	13.1 ± 0.2^{a}	7.4 ± 0.2^{a}	6.4 ± 0.2^{a}		F : [82]
53	*Vernonia amygdalina*	IL4032	1.5 ± 0.1	1.4 ± 0.2	13.1 ± 0.2^{a}		F : [114]

F: leaf, Fr: fruit, Flr: flowers, T: stem, R: roots, PA: aerial parts, PE: whole plant, G: gum, The extracts are compared to the positive control which is ascorbic acid and the level of significance of the difference is expressed by letters a, b and c: a $p<0.01$, b $p<0.001$, c $p<0.0001$.

Antioxidant activity screening results show that 73.3% of extracts have antioxidant activity and the most active 5/11 extracts are leaf. The only 8% of plants that showed strong antioxidant activity are: *Bidens pilosa* (leaves and roots), *Dalbergia katangensis* (Leaves), *Dialium angolense* (leaves), *Senecio cineraria* (aerial parts), *Solanecio cydoniifolius* (aerial parts) and *Vernonia amygdalina* (leaves and roots) (figure 4).

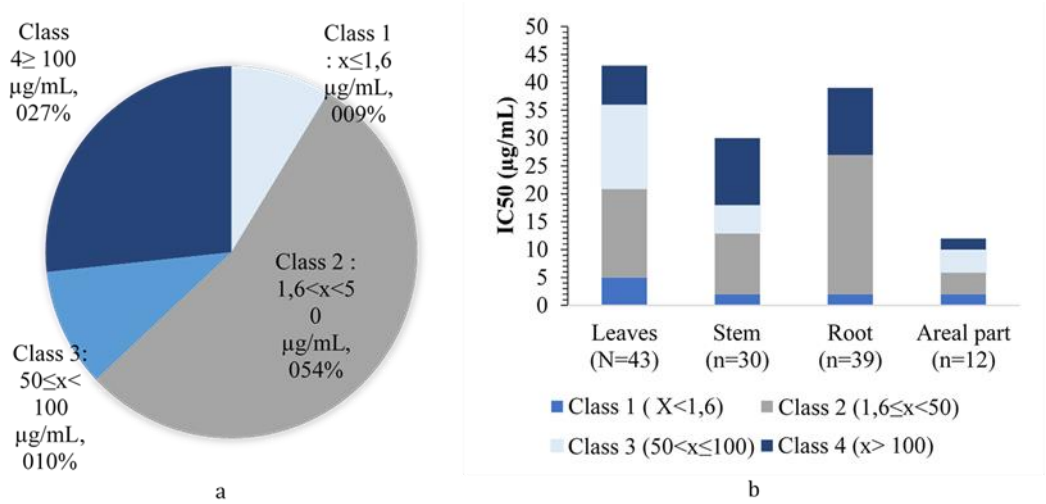

Figure 4 Distribution of extracts according to their IC_{50} values for the whole antioxidant screening (a) and for each organ (b).

4. Discussion

During this study, 53 plants selected from an ethnobotanical survey carried out on plants known to be antimalarial in Bagira in eastern DRC were studied. This study focused on the search for secondary metabolites in various organs of these plants, and the demonstration of the antioxidant potential of methanolic extracts from their organs used as antimalarial drugs in Bagira. The interest in evaluating the antioxidant potential of antimalarial substances comes from the fact that plants with antioxidant potential could prevent the oxidative stress which occurs in malaria disease. As for the phytochemical screening of secondary metabolites, it constitutes the first step towards the isolation and characterization of the active compounds.

This study reports, for the first time, the phytochemical knowledge of 11 plants, *Aframomum laurentii*, *Clematis villosa*, *Crassocephalum montuosum*, *Crassocephalum picridifolium*, *Dalbergia katangensis*, *Dialium angolense*, *Isoberlinia angolensis*, *Isoberlinia tomentosa, Julbernardia paniculata, Rothmannia engleriana and Solanecio cydoniifolius*. Among these plants, *Aframomum laurentii* and *Dialium angolense* with 8 phytochemical groups each, *Julbernardia paniculata* as well as *Rothmania engleriana* with 7 phytochemical groups each, can be considered as the 4 most interesting species from a phytochemical point of view because of their diversity in secondary metabolites.

The results of this study show that 30 of these 53 plants are already known from a phytochemical point of view although no antimalarial molecules have been reported (Table 1). This study confirmed previous results for some of these species. This is the case with flavonoids and terpenoids in *Chenopodium opulifolium* [45] or flavonoids and terpenoids in *Ekebergia benguellensis* [51]. Furthermore, some previous phytochemical knowledge has not been confirmed by this study. This is the case of the species *Ekebergia benguelensis* where we did not find coumarins in the root bark and yet previously 4-methoxy-5-hydroxymethylcoumarin had been isolated there [50]. The fact that the plants were not harvested in the same environment is a likely explanation for these observed disparities given that the phytochemical composition of the plant in secondary metabolites depends on several factors such as climate, age of the plant or the place of harvest [115]. It could also be varieties different from those studied previously. It would therefore be interesting to carry out a simultaneous study between these different specimens to have a clear point of view.

Terpenoids and flavonoids were the most frequent alongside several metabolites with antiplasmodial and antioxidant potential (Table 1 and Figure 3). Note that several studies have reported the preponderance of these metabolites among phytochemical groups with antimalarial potential [21,22]. The identification of these phytochemical groups within these 53 plants could constitute a first orientation for a more in-depth screening possible mainly on the 11 plants which were until then little known from a phytochemical point of view.

Only 18 plants (32%) have never been assessed for prior antioxidant activity (table 2). These spacies are, *Aframomum laurentii, Bobgunia madagascariensis, Chenopodium opulifolium, Clematis villosa, Crassocephalum montuosum, Crassocephalum picridifolium, Dalbergia katangensis, Dialium angolense, Dialopsis africana, Ekebergia benguellensis, Flueggea virosa, Hypoestes triflora, Isoberlinia angolensis, Isoberlinia tomentosa, Julbernardia paniculata, Ochna*

schweinfurthiana, Rothmannia engleriana and *Solanecio cydoniifolius*. The results obtained for these plants constitute the first information concerning their antioxidant potential. In this category of plants, only *Dalbergia katangensis, Dialium angolense* and *Solanecio cydoniifolius*, with IC_{50s} ≤1.6 µg / mL, are very active and of which, *Dialium angolense* is the only species which has 8 phytochemical groups. These 3 plants could constitute interesting candidates for a more in-depth antioxidant investigation.

Among the 18 plants so far unrecognized from the point of view of antioxidant activity, 11 are also unknown from the phytochemical and antimalarial point of view [116]. These species are *Aframomum laurentii, Clematis villosa, Crassocephalum montuosum, Crassocephalum picridifolium, Dalbergia katangensis, Dialium angolense, Isoberlinia angolensis, Isoberlinia tomentosa, Julbernardia paniculata, Rothmannia engleriana* and *Solanecio cydoniifolius*. Over 80% of these plants contain coumarins (100% of plants) and terpenoids (81.2% of plants). However, it has been previously reported that coumarins [114,117] and terpenoids [118,119] constitute true groups of natural antioxidants. Their frequent presence in the plants could therefore constitute an explanation of the antioxidant activity demonstrated in the extracts examined. On the other hand, the fact that 68% of plants have already been investigated for antioxidant activity suggests that there is a high probability of encountering in the 32% of plants not studied, interesting antioxidant compounds.

5. Conclusion

This study highlights for the first time the antioxidant activity of 18 plants in which several phytochemical groups with both antioxidant and antimalarial activity such as flavonoids and terpenoids are frequently found. It shows that among these plants, 11 are also unrecognized from the phytochemical antioxidant and antimalarial point of view. She suggests that these plants, such as *Dalbergia katangensis, Dialium angolense* and *Solanecio cydoniifolius*, whose antioxidant activity has just turned out to be interesting, be further investigated in the hope of discovering compounds that are both anti-free radicals and antimalarial.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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