Phenolic content and antioxidant activity of some wild plant teas of Masako

Célestin Maando BAMAWA*[1], Léopold Mianda NDJELE[2] and François Mazibo FOMA[3]

[1]Département de Chimie, Université de Kisangani, Kisangani, RD. Congo

[2]Département d'Ecologie et de Gestion des ressources végétales, Université de Kisangani, Kisangani, RD. Congo

[3]Département de Chimie et Industries Agricoles, Institut Facultaire des Sciences Agronomiques (IFA), Yangambi, RD. Congo

E-mail: celestinbamawa@yahoo.fr, Tel : 00243998824010

ABSTRACT

Human body produces free radicals which can damage biomolecules and cause diseases. Phenolic compounds of foods can prevent those damages. The aim of this study is to identify some phenolic wild plant teas of Masako and evaluate the phenolic content and the antioxidant activity of their aqueous decoctions.

Some wild plants consumed as teas were collected in Masako. Their chemical screening was done for identifying plants which contain phenolic compounds. Aqueous decoctions of phenolic plants were prepared and their phenolic content was determined by UV-Vis Spectrophotometry. *In vitro* antioxidant activity (Reducing and Scavenging power) of these decoctions was evaluated as well.

Leaf aqueous decoctions of *Alchornea cordifolia, Sabicea johnstonii, Hymenocardia ulmoides* and *Ipomoea involucrata* contain polyphenolic compounds. These decoctions contain significant amount of polyphenolic compounds and show significant reducing and scavenging power.

These aqueous decoctions are other potential sources of natural antioxidants which could prevent diseases associate to oxidative stress.

Key words: Antioxidant activity, Chemical screening, Phenolic content, Wild plant tea.

1. INTRODUCTION

Reactive oxygen species (ROS) have many physiological functions such as human defenses against undesirable microorganisms and intracellular signalizations. Their production is physiological controlled by intracellular system of defenses constituent with enzymes and different antioxidants [2,3,6,17].

The overproduction of free radicals by biochemical and physiological human processes cause biological molecule damages which are responsible for various diseases such certain forms of cancer, cardiovascular and neurodegenerative diseases [2,7].

Antioxidant compounds present in food such phenolic compounds could react with free radicals and prevent these damages. Several investigators reported that consumption of polyphenol-rich foods and beverages is associated with a reduced risk of diseases associated to oxidative stress [19,20].

Masako, forest reserve about 14 Km from Kisangani (DR Congo), is rich in vegetal biodiversity. Masako forests are in the category of equatorial evergreen rainforests. Many ethno-botanical studies such researches of [16] have identified the wild edible plants (WEP) of Masako region.

Wild plants of this region, consumed as teas, have not been studied for their phenolic content and antioxidant activity. They could contain phenolic compounds which contributed to their oxidant activity. The objective of this study was to identify some phenolic wild plants of Masako consumed as teas, to quantify the phenolic compounds and to evaluate the antioxidant activity of their aqueous decoctions.

2. MATERIAL AND METHODS

2.1. Plant materials and aqueous extracts

Fresh Plant materials were collected in Masako (14 km from Kisangani, DR Congo) and were identified at the Faculty of Sciences of the University of Kisangani.

Sample (0.5 g) was boiled in 50 ml of distilled water for 20 min. Then extracts were filtrated through Whatman paper, diluted with distilled water to 100 ml and stored in the dark in freezer until use.

2.2. Humidity of plant materials and chemical screening of decoctions

Drying sample at 105°C was used as method of determination of humidity. The following tests were used for detection of some phytoconstituents and undesirable compounds in the WEP decoctions: $FeCl_3$ test for detection of polyphenols, gelatin test for detection of tannins, cyanidine test for detection of flavonoids , decoloration or bleaching test for detection of anthocyanins, tests with Mayer and Dragendorff reagents for detection of

alkaloids, foam test for detection of saponins, $CaCl_2$ test for detection of oxalates, diphenylamine test for detection of NO_3^- and $AgNO_3$ test for detection of CN^- and NO_2^-.

2.3. Quantification of phenolic compounds

2.3.1. Total phenolic content

Folin-Ciocalteu reagent (FCR) was used to quantify phenolic compounds of leaf decoctions, the assay described by [8,13] was adapted. Briefly, 0.3 ml of the decoction and 0.5 ml of methanol were mixed with FCR (0.7ml) and left to stand for 5 min at room temperature. 0.7 ml of Na_2CO_3 10% was then added. After incubating in dark at room temperature for 90 min, the absorbance was read at 760 nm using UV-Vis spectrophotometer. The total phenolic content was determined as Gallic acid from a calibration curve. The following equation was used:

$$mg\ GAE/l = (A/m) \times f_D \times f_{P'}$$

A, m, f_D and $f_{P'}$ were absorbance, slope of calibration curve, dilution and proportionality factors respectively.

2.3.2. Total tannin content

The precedent assay used to determine total phenolic content was adapted to estimate Total tannin. The differential absorbance, before and after precipitation of tannins with gelatin ($10\%_{W/V}$), was used to determine total tannin as Tannic acid from a calibration curve. The sample used was first diluted with methanol. The following equation was used:

$$mg\ TAE/l = (A/m) \times f_D \times f_{P'}$$

2.3.3. Total flavonoid content

The assay described by [12,22] was adapted for the analysis of total flavonoid. Briefly, 0.3 ml of decoction was mixed respectively with methanol (0.7 ml), distilled water (0.3 ml) and 0.1 ml of $NaNO_2$ (5%), and then mixed. After 5 min, 0.1 ml of $AlCl_3$ (10%) was added and mixed. After 5 min 0.5 ml of NaOH (1 mM) was added and sample absorbance was read at 510nm using UV-vis spectrophotometer. The total flavonoid content was determined as quecetin from a calibration curve. The following equation was applied:

$$mg\ QuE/l = (A/m) \times f_D \times f_{P'}$$

2.3.4. Total anthocyanin content

The method described by [11,14] was adapted for the analysis of total anthocyanin. Briefly, one drop of HCl (conc) was added to 4 ml of the leaf decoction and then 100µl of disulfite ($10\%_{W/V}$) or distilled water. After incubating in dark at room temperature for 15 min, the absorbance was read at 530 nm using UV-Vis spectrophotometer. The change of absorbance was used to determine total anthocyanin, as cyanidin-3-glucose, according the following formula:

$$mg\ C3GE/l = (A/\varepsilon \times l) \times Mw \times V \times f_D \times f_{P'}$$

A, ε, l, Mw and V were absorbance, molar extinction coefficient, path length of the microcuvette used, molecular weight and volume respectively.

2.4. Reducing and scavenging power of decoctions

2.4.1. Ferric reducing power

The assay described by [21,22] was adapted to estimate ferric reducing power. Briefly, 0.5 ml of the decoction, in different concentrations, was mixed with 0.5 ml of phosphate buffer (0.2 M, pH = 6.6) and 0.5 ml of potassium ferricyanide (1%). The mixture was reacted in a 50°C water-bath for 20 min, then cooled at room temperature, and mixed with 0.5 ml of trichloroacetic acid (10%) for 5 min. 1 ml of the supernatant mixing with 1 ml of distilled water and 0.1 ml of $FeCl_3$ (0.01%) was left to stand for 10 min. The absorbance was then read at 700 nm and used as the reducing power indicator.

2.4.2. DPPH free radical activity

The assay described by [1,11] was adapted to estimate DPPH free radical activity. Briefly, 2 ml of DPPH (0.1786 mM in methanol) was added to 100µl of decoction in different concentration. After incubation of 20 min in the dark at room temperature, the absorbance was measured at 517 nm. The DPPH radical scavenging activity was calculated according to following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = (A_0 - A_m) \times 100/A_0$$

A_0 and A_m were absorbance of control and absorbance of tested decoction respectively.

3. RESULTS AND DISCUSSION

3.1. Chemical screening of decoctions

Chemical screening of six wild plant decoctions consumed as teas were done; the results of these investigations are reported in Table 1.

The results of screening, presented in table 1, showed that four leaf decoctions contain phenolic compounds including flavonoids. *H. ulmoides* and *I. involucrata* contain also anthocyanins, tannins are only present in *A. cordifolia*. [7,15] reported the presence of polyphenolic compounds (flavonoids and tannins) in the aqueous or methanolic extracts of *A. cordifolia* collected in Dschang (Cameroon) and Kinshasa (DR Congo). [7] reported the presence of saponins and the absence of alkaloids in *A. cordifolia* leaf decoction, which agrees with our results.

Both aqueous decoctions of *S. johnstonii* and *I. involucrata* contain saponins. $C_2O_4^{2-}$, CN^-, NO_2^- and NO_3^- are absents in aqueous decoctions of phenolic plants investigated.

3.2. Quantification of phenolic compounds

Phenolic content of four leaf decoctions was determined by UV-Vis Spectrophotometry; the results of these analyses are reported in Table 2.

As shown in Table 2, *A. cordifolia* decoction contained the highest phenolic and flavonoid contents, followed by *S. johnstonii.* These results revealed that *I. involucrata* or *H. ulmoides* contained the lowest phenolic or flavonoid content respectively.

The amount of phenolic compounds was found to be more in decoctions of *A. cordifolia*, *S. johnstonii* and *H. ulmoides* compared to some juices such as blueberry and grape juice of *Morinda citrifolia* (256.4 mg GAE/l and 60.1 mg GAE/l) analyzed by [4] and some wines such as Posip (292 mg GAE/l) or Marastina (402 mg GAE/l) analyzed by [11] and Debina (261 mg GAE/l) or Athiri (213 mg GAE/l) analyzed by [19].

Phenolic content of these decoctions were lower than phenolic content of some wines or juices such Limnio (2704 mg GAE/l) or Syrah (3184 mg GAE/l) analyzed by [19], *Citrus lemon* (600 GAE/l) or *Citrus aurantium* juice (532 GAE/l) analyzed by [18] and Strawberry juice (1271.85 GAE/l) analyzed by [9] and red wine BoCS (3200 mg GAE/l) analyzed by[14].

The flavonoid content of some of these decoctions were higher than the content of Strawberry (205.98 QuE/l) and Red raspberry juice (217.39 QuE/l) analyzed by [9] and some wines such as Limnio (174 QuE/l) or Debina (35 QuE/l) analyzed by [19] and red wine BoCS (318 mg QuE/l) or GaTA (5.56 mg QuE/l) analyzed by [14].

It was found that anthocyanin content of *I. involucrata* and *H. ulmoides* decoctions were lower than anthocyanin content of some wine such as red wine BOCS (10.8 mg/l) and Badié (222 mg/l) analyzed by [11] and [14] respectively.

Leaf decoctions investigated were free alcohol and free alkaloids including caffeine, their regular consumption might be helpful in human body for their phenolic compounds.

3.3. Reducing and scavenging power of decoctions

3.3.1. Ferric reducing power

The ability of decoction to reduce Fe (III) of ferricyanide in vitro was used to evaluate his reducing power, absorption at 700 nm revealed the presence of reductants in the leaf decoctions. Fig. 1 shows the ferric reducing power of *A. cordifolia*, *S. johnstonii*, *H. ulmoides* and *I. involucrata* leaf aqueous decoctions.

The greatest reducing power was observed in *A. cordifolia* decotion. As shown in Fig.1, the ferric reducing power increased with the phenolic concentrations of these decoctions. Statistical analysis showed that these concentrations are correlated positively with the reducing power. Relation coefficient was 0.89742, 0.96843, 0.94364 and 0.98283 for *A. cordifolia*, *H. ulmoides*, *S. johnstonii* and *I. involucrata* respectively. This means that the ability of these leaf decoctions to reduce iron (III) is mainly due to their phenolic compounds. [11] and several investigators reported good correlation between total phenols and ferric reducing power. The reducing power of a compound is due to its electron transfer ability.

The reducing power obtained *in vitro* showed that phenolic compound of these leaf decoctions could react with free radicals in human body, by transferring electron to them, and then reduce the risk of oxidative biological molecule damages.

3.3.2. DPPH free radical activity

In biological systems, biomolecules damages are caused by overproduction of free radicals. DPPH free radical is widely used *in vitro* to evaluate the scavenging power of extracts. Fig. 2 shows the scavenging power of *A. cordifolia*, *S. johnstonii*, *H. ulmoides* and *I. involucrata* leaf aqueous decoctions.

As showed in Fig. 2, the inhibition of DPPH increased with the phenolic concentrations of decoctions. Statistically, there is a positive correlation between phenolic concentrations and inhibition of $DPPH^{\bullet}$. Relation

coefficient was 0.89628, 0.93298, 0.99557 and 0.98422 for *A. cordifolia*, *H. ulmoides*, *S. johnstonii* and *I. involucrata* respectively. This also means that the ability of these leaf decoctions to scavenge DPPH• is mainly due to their phenolic compounds. [10] and other researchers reported that the scavenging power of phenolic compounds is due to their hydrogen atom donating ability.

As shown in Fig. 2, the capacity of the leaf decoctions to scavenge 50% of DPPH• ranged from 60 to 206 µg GAE/ml. The observed value for *A. cordifolia* decotion (60 µg GAE/ml) was lower than the value of *I. involucrata* (80 µg GAE/ml) and *S. johnstonii* (204 µg GAE/ml) or *H. ulmoides* (206 µg GAE/ml). These results indicate that *A. cordifolia* decoction has the highest capacity to scavenge DPPH• followed by *I. involucrata*. *H. ulmoides decoction* presented the lowest scavenging capacity.

Phenolic compounds that are observed in our leaf decoctions were able to scavenge free radicals in vitro, this means that these phenolic compounds could reduce the amount of free radicals in the human body and then prevent bimolecule damages which are responsible for various diseases.

4. CONCLUSION

The results of this study showed that queous decoctions of *A. cordifolia*, *S. johnstonii*, *H. ulmoides* and *I. involucrata* contain phenolic compounds and are healthy useful for their antioxidant activities.

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TABLES

Table 1. Detection of some phytoconstituents and undesirable compounds in decoction

Legend: Po, Fl, An, Ta, Al, Sa, Ox, Cy, Ni, Nt, Le, Sb, St and * mean phenolics, flavonoids, anthocyanins, tannins, alkaloids, saponins, oxalates, cyanides, nitrites, nitrates, leaves, stem bark, stem and not done respectively

N°	Scientific name	Botanical family	Part used	Chemical screening of decoction									
				Po	Fl	An	Ta	Al	Sa	Ox	Cy	Ni	Nt
1	*Alchornea cordifolia*	Euphorbiaceae	Le	+	+	–	+	–	–	–	–	–	–
2	*Hymenocardia ulmoides*	Hymenocardiacea e	Le	+	+	+	–	–	–	–	–	–	–
3	*Sabicea johnstonii*	Rubiaceae	Le	+	+	–	–	–	+	–	–	–	–
4	*Ipomoea involucrata*	Convolvulaceae	Le	+	+	+	–	–	+	–	–	–	–
5	*Croton haumanianus*	Euphorbiaceae	Sb	–	–	–	–	*	*	*	*	*	*
6	*Setaria megaphylla*	Poaceae	St	–	–	–	–	*	*	*	*	*	*

Table 2. Content of Phenolics, Flavonoids, Tannins and Anthocyanins of leaf decoctions

Legend: H, GAE, QuE, TAE, C3GE and * mean humidity of fresh leaves, gallic acid equivalent, quercetin equivalent, tannic acid equivalent, cyanidin-3-glucose equivalent and absence respectively

N°	Scientific name	%H	mg GAE/l	mg QuE/l	mg TAE/l	mg C3GE/l
1	*A. cordifolia*	66.0725	485.5981	281.0873	171.4859	*
2	*H. ulmoides*	83.2927	433.6788	129.3339	*	1.0672
3	*S. johnstonii*	48.7233	456.2790	211.8736	*	*
4	*I. involucrata*	71.8460	184.7704	150.9681	*	0.8457

FIGURES

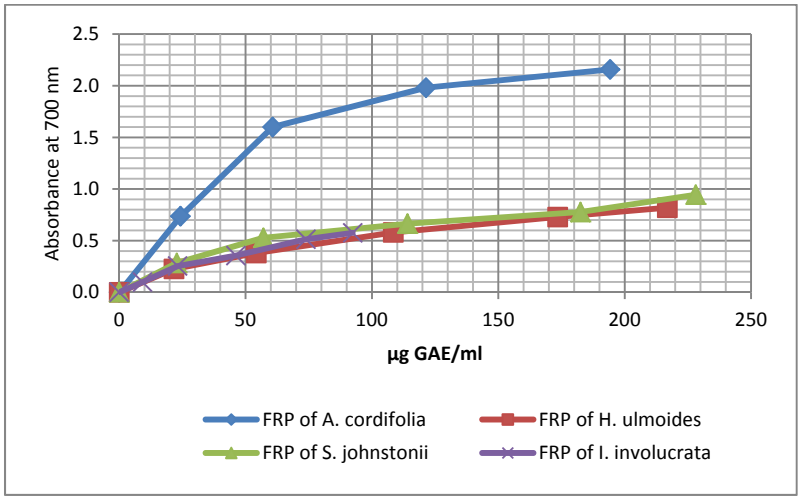

Fig. 1. Ferric reducing power (FRP) of leaf aqueous decoctions

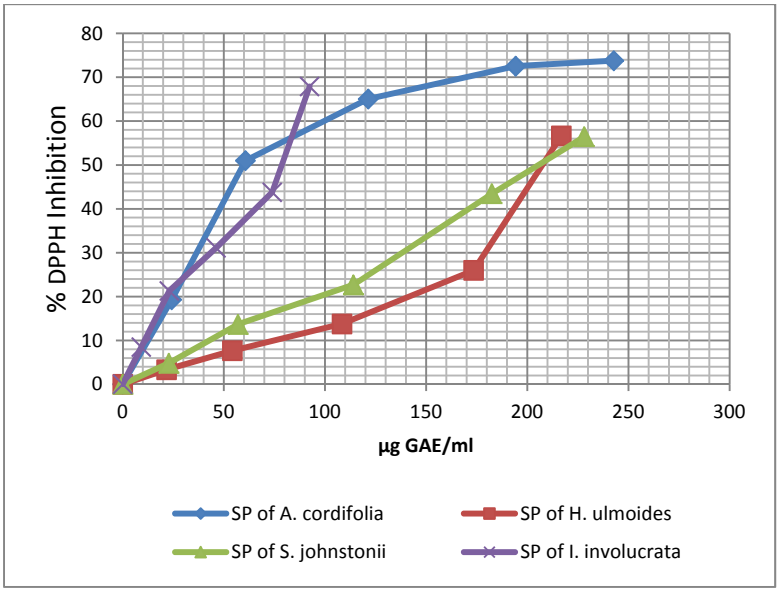

Fig. 2. Scavenging power (SP) of leaf aqueous decoctions