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ANTIOXIDANT PROFILES OF ALCOHOLIC TINCTURES FROM *HETEROTIS ROTUNDIFOLIA* (SM.) JACQ.-FÉL. (MELASTOMACACEAE) BY DPPH RADICAL TRAPPING

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ABSTRACT
Phytophenols are widely distributed in the vegetable kingdom. This is the reason why, for the first time, we evaluate the phenolic compound content and the antioxidant profile *Heterotis rotundifolia* alcoholic tinctures, a therapeutic plant species from Côte d'Ivoire. The study takes into account the extraction, the total phenol quantification and flavonoid contents as well as that of the antioxidant activity vis-a-vis the stable radical DPPH by the median effective concentration (EC_{50}). The decoction provides the best yields of extracts. Overall, alcoholic tinctures showed variable total phenol and flavonoid contents and notable antioxidant profiles. The results obtained allow affirming that *Heterotis rotundifolia* is an antioxidant plant.

KEYWORDS: *Heterotis rotundifolia*, phytochemistry, antioxidant activity, EC_{50}.

INTRODUCTION
The Melastomataceae family includes dicotyledonous species containing at least 4400 species divided into 180 kinds. Herbaceous plants, shrubs, trees and / or epiphytic lianas populate this family, and some species are commonly used in native medicinal practice. *Heterotis rotundifolia* from Côte d'Ivoire known as "Hindjimpè" in dialect Akyé, is a hairy-stemmed herb, rooting at nodes, high or long 30-40 cm. It is a species of the humid forest regions. Its isolated terminal flowers (4 cm in diameter), are pink or pale purple. The plant grows on the slopes, on wastelands, and in ancient crops.[1,2] In Côte d'Ivoire, its leaves are prized for its many traditional medicinal uses.[1] To our knowledge, the various sources of information drawn from the literature indicate that this plant has never been studied. In this work, we report for the first time the phytochemical composition and antioxidant potential of *Heterotis rotundifolia* dyes. This should make it possible to highlight its therapeutic virtues, for the purpose of its valorization and to accredit its usefulness in indigenous therapy.

MATERIAL AND METHODS
Tinctures obtainment
The leaves, stems and roots of the plant were harvested in Abidjan, on the site of the university Nangui Abrogoua (5 ° 23 '21 "N, 4 ° 01' 09" W) in June 2017. After authentication of the plant at the National Center of Floristic (NCF) located at the university Felix Houphouët-Boigny (Cocody-Abidjan), following the available herbarium (reference Malan 552), a specimen was kept in the laboratory. After cleaning with water, the organs were dried under air conditioning (16°C) for 5 days and then reduced to powder. The powder (40 g) of each plant sample (plant, leaves, stem + roots, leaves + stem + roots) was previously treated with hexane (150 ml) in a Soxhlet extractor to remove lipids and chlorophylls.

Alcoholic decoction°: The each sample residue (5 g) in ethanol (50 ml, EtOH, 96%) was boiled for 15 min. After filtration under vacuum, tinctures (Table 1) were concentrated under reduced pressure at 59°C.

Alcoholic maceration°: The powder of each sample (5 g) was macerated in EtOH (96%, 50 ml) at 26°C under constant agitation during 2 h. After filtration under vacuum, tinctures (Table 1) were concentrated under reduced pressure at 59°C.

Table 1: Tinctures coding by extraction type and by plant sample.

Sample	Plant	Leaves	Stem+Roots	Leaves+Stem+Roots
Tinctures D	DP	DF	DTR	DFTR
Tinctures M	MP	MF	MTR	MFTR

D and M tinctures obtained respectively by decoction and maceration.

Phytophenol groups quantification

Total phenols were determined according to Folin-Ciocalteu[3,4] method using gallic acid as standard. Total flavonoids quantification was carried out according to the method of Hariri[5] modified by Konan.[6] Quercetin was the standard used.

DPPH radical scavenging assay

The method described by Espin[7] modified by Sladjana[8] was used to measure the antioxidant activity of tinctures vis-a-vis the stable radical DPPH (2.2-diphényl-1-picrylhydrazyl). Vitamin C, quercetin and gallic acid were the standards used as positive control. The DPPH percentage reduction was calculated as follows: % DPPH Reduction (PR) = $[1 - (A_0 / A_1)] \times 100$

A_0 and A_1 are the absorbance of the measured aliquot at times t and control, respectively.

The efficacy index of the antioxidant activity was expressed in EC_{50} (median effective concentration at times t), which is the ratio of CR_{50} (concentration that reduces 50% of DPPH) to the DPPH initial concentration.

RESULTS AND DISCUSSION

Phytophenols content

The extractions yields vary from one extract to another, according to the plant matrix (Table 2).

Table 2: Extractions yields.

Tinctures	MP	DP	MF	DF	MFTR	DFTR	MTR	DTR
Mass	0.38	0.44	0.24	0.22	0.23	0.45	0.38	0.43
Yiels (%)	7.6	8.8	4.8	4.4	4.6	9	7.6	8.6

D and M tinctures obtained respectively by decoction and maceration.

The tinctures provided by decoction have higher yields than those obtained by maceration, with the exception of DF. Indeed, the decoction required a boiling time of the plant in ethanol. The thermal activation has therefore caused on the one hand, the solvent's agitation and the vegetable matrix, and on the other hand, has further increased the solubility of the active ingredients and their diffusion in the solvent.

The linear regression curve of gallic acid ($y = 10^{-3}x + 398\ 10^{-4}$ ($R^2 = 0.9925$)) allowed to quantify (µg g^{-1}) the total phenols contained in the *H. rotundifolia* tinctures. The total flavonoids content (FT) was determined from FT percentage, according to the equation°:

% FT = $(0{,}05 \times A_{ext}/A_q) \times 100 \times d/C_{ext}$, or A_{ext} and A_q are respectively the absorbance of the sample and quercetin; C_{ext} and d are the concentration of the sample (mg ml^{-1}) and the dilution factor respectively; converted (µg g^{-1}) to the amount of dry matter in the sample. The phytochemical screening results are shown in Fig 1.

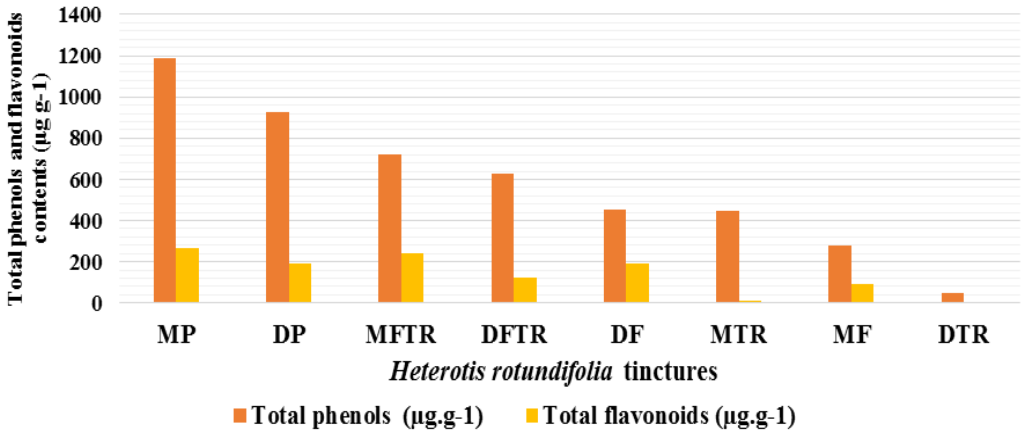


Figure 1: Total phenols and flavonoids contents in the tinctures.

It appears that tinctures have variable total phenols and flavonoids contents, which are respectively 1.28; 1.15; 1.01; 5.99 times higher. Thus, the maceration is the most efficient extraction's method for obtaining alcoholic tinctures enriched in total phytophenols compared to the decoction. This accredits the thermo sensitivity of the

phenolic plant principles. Moreover, these results show a phenolic secondary metabolites irregular distribution in the plant. MP tinctures has high total phenols and flavonoids contents. The phytophenols solubility depends on their structural diversity, which conditions their extractability.[9,10] The figure 1 indicates that MP tincture is rich in phenolic and flavonoic phytocompounds, which seems to attribute to *H. rotundifolia* an antioxidant activity. The active principles plant have considerable effects on nutrition and human health.[11]

Tinctures antioxydant activity

A comparative study of anti-radical activity of HR tinctures vis-a-vis the stable radical DPPH was evaluated by spectrophotometric method. Vitamin C (ascorbic acid), gallic acid (phenol acid) and quercetin (flavonol) were used as antioxidant standards. The results were indicated in the Fig 2 and 3.

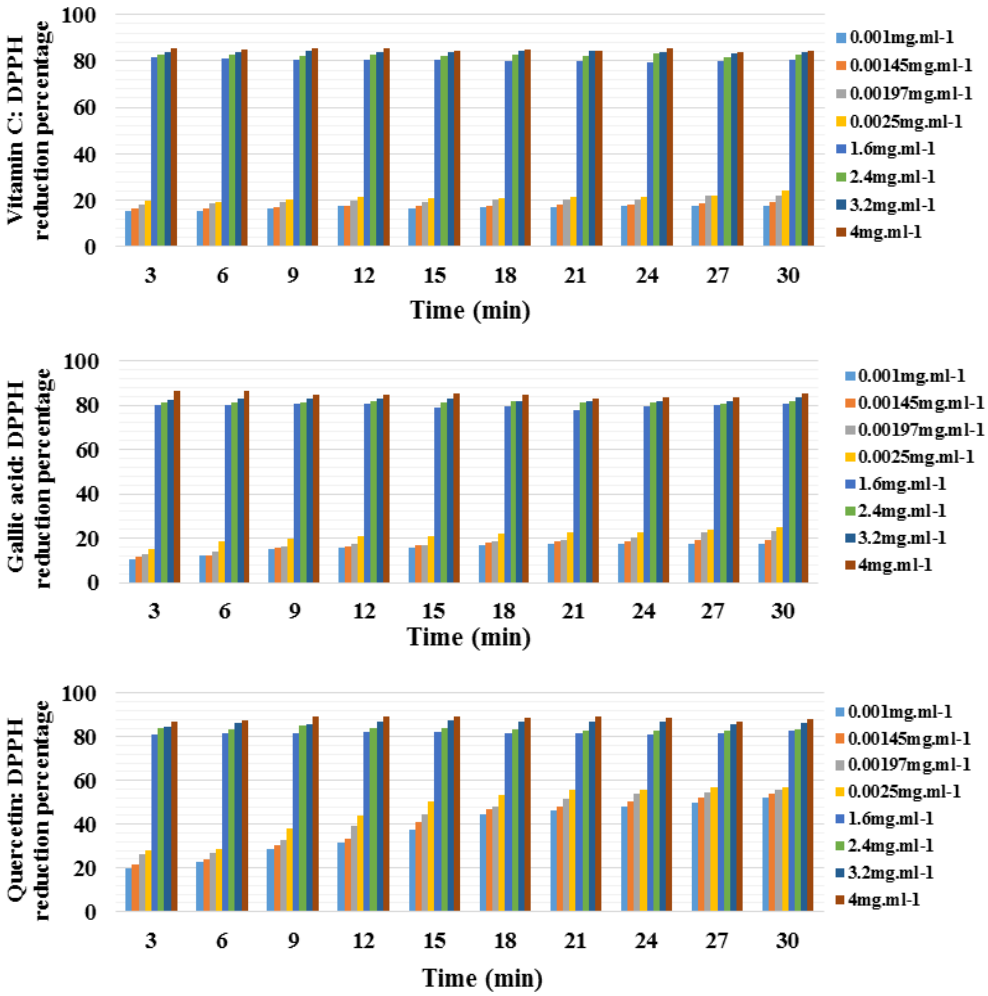
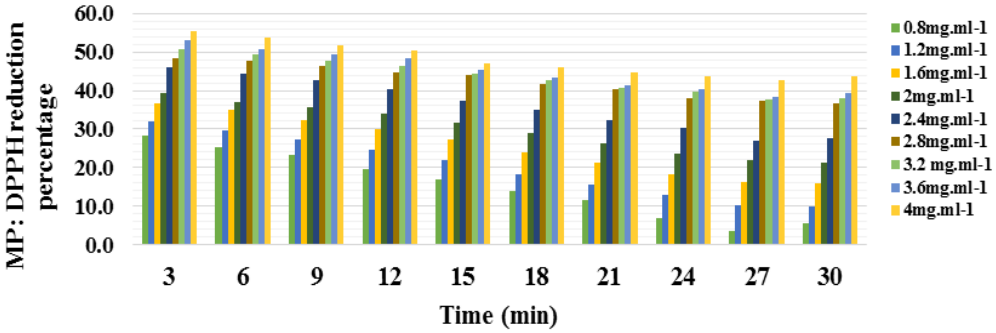


Figure 2: Anti-radical profiles of vitamin C, gallic acid and quercetin against DPPH.



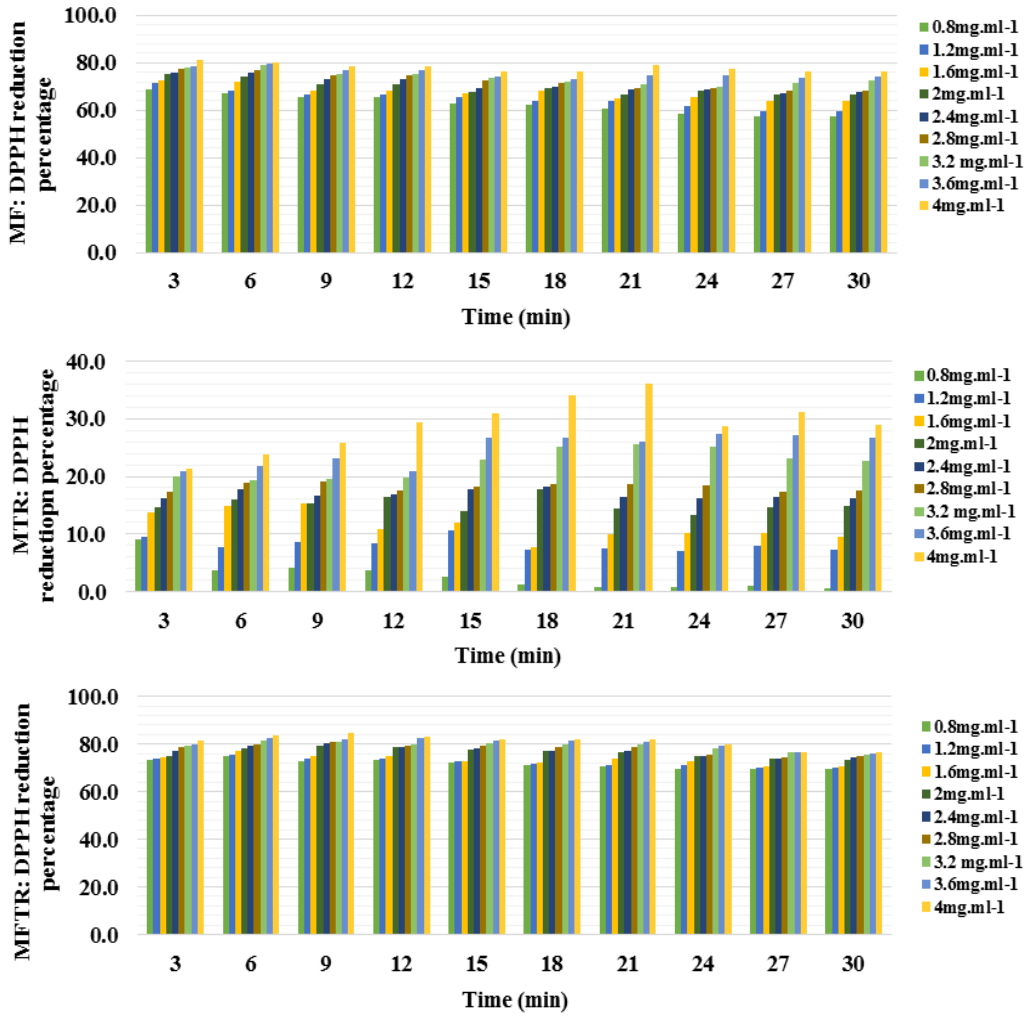
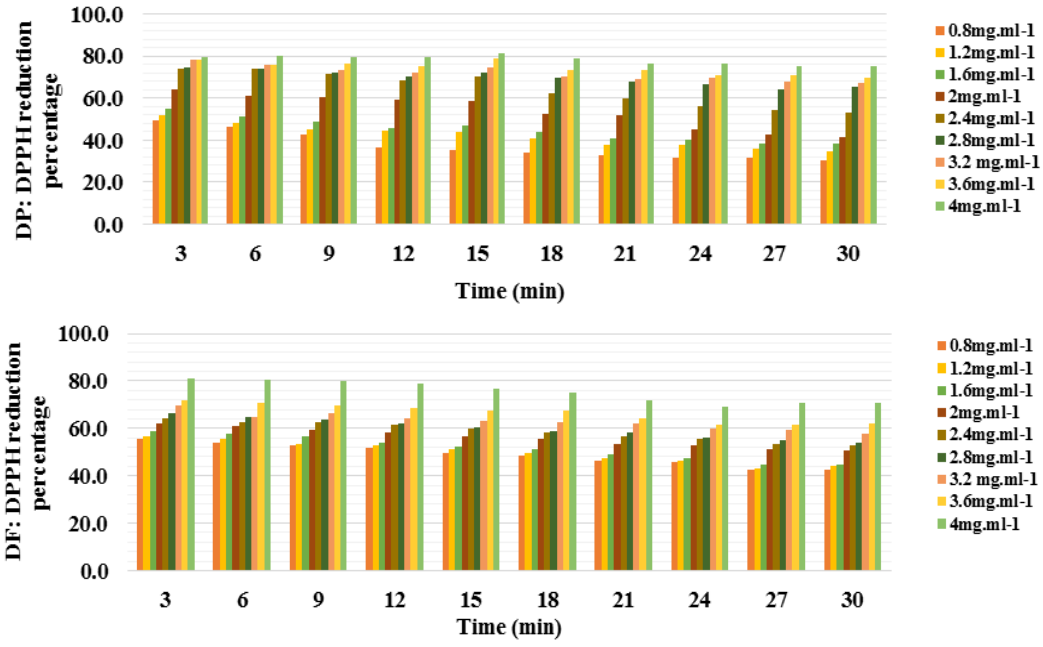


Figure 3: Anti-radical profiles of *H. rotundifolia* tinctures obtained by maceration.



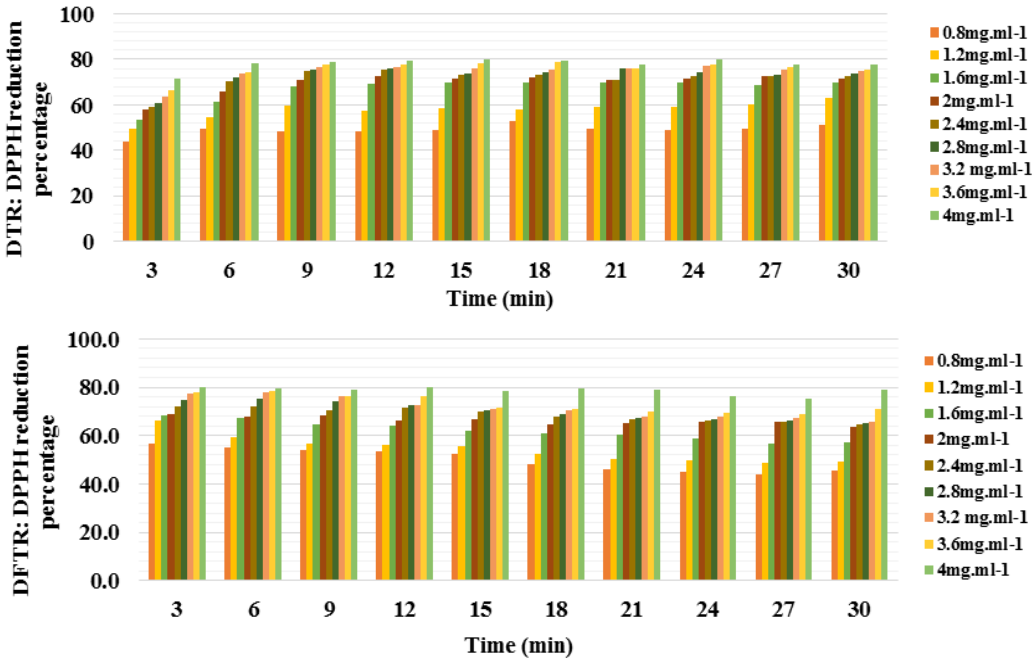


Figure 3: Anti-radical profiles of *H. rotundifolia* tinctures obtained by decoction.

The Figure 3 shows the maximum antioxidant profiles of approximately 80% at 4 mg ml^{-1} and minimal of 17.33; 17.77 and 52% at 0.001 mg ml^{-1} respectively for vitamin C, gallic acid and quercetin. The structure-activity relationship (Fig. 4) attests to these results.[12,13] So, we demonstrate, with regard to reference antioxidants, that all the analyzed tinctures exhibit a proven antioxidant effect concentration-dependent against DPPH. What obliges us to believe that *H. rotundifolia* antioxidant behavior would be due to its phenolic composition (Fig. 1), and it is true, by a fast kinetics (hydrogen donation of OH group by some phytophenols).[14]

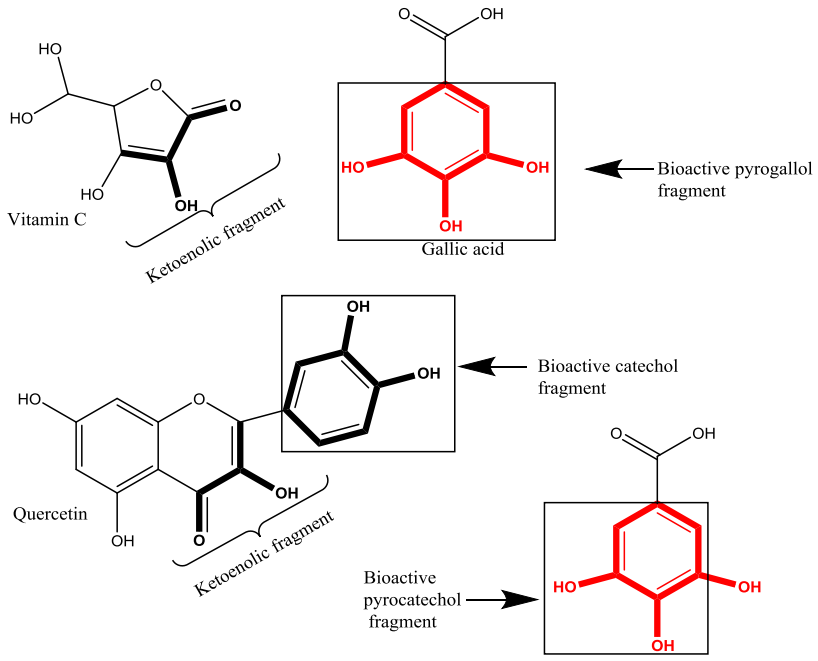


Figure 4: Molecular structures of vitamin C, gallic acid and quercetin.

The method of obtaining of the tinctures makes observe a fluctuation in their antioxidant behavior towards the DPPH. So, by referring to results (Fig. 3) according to the time required of more than half DPPH reduction, we can classify *H. rotundifolia* tinctures in categories A, B and C, respectively for fast antioxidant activity (at least 80% of the DPPH trapping in 3 min), intermediary (80% of the DPPH trapping in 15 min) and slow (less than 50% of the DPPH trapping after 15 min) (Fig. 3). However, MP tincture can be categorized as A because it caused the DPPH maximal trapping of 55.52% in 3 min to stabilize then. Overall, these results let think that the

maximal DPPH reduction takes place in 3 min. In addition, the percentages variability of the DPPH maximal reduction seems to be dependent not only in the heterogeneous composition of the active phenolic principles, which contain the alcoholic tinctures, but also in their synergic or antagonistic action.[14]

Table 3: *H. rotundifolia* tinctures classification in categories based on percentage reduction of DPPH.

Categorie	Tincture	DPPH reduction percentage
A	MF	81.28
	MFTR	81.28
	DF	80.82
	DP	80.38
	DFTR	80.00
B	DTR	79.68
C	MTR	36.09

D and M tinctures obtained respectively by decoction and maceration.

An appreciation of the efficiency of the DPPH reduction by the alcoholic tinctures was highlighted by the EC_{50} determination (Table 3), which translates the median concentration of a compound, which reduces the DPPH.[15] The lower this parameter, the better the compound has a good antioxidant activity.

Table 4: EC_{50} of tinctures and reference antioxidants.

	Time (min)									
Extract	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**27**	**30**
Vit C	11.67	12.23	12.33	12.33	12.23	12.33	12.33	12.33	12.23	12.23
AG	15.00	13.90	12.77	12.77	12.77	12.77	12.77	12.77	12.67	12.77
QTN	10.567	10.567	8.900	7.233	5.000	1.667	0.557	0.217	0.167	0.033
MP	101.00	108.33	118.33	129.00	133.33	133.33	133.33	133.33	133.33	133.33
DP	31.10	50.00	53.33	54.00	55.67	61.67	65.00	71.00	74.33	76.00
MF	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67
DF	26.67	26.67	26.67	26.67	26.67	39.33	54.00	57.67	71.67	74.33
MTR	133.33	133.33	133.33	133.33	133.33	133.33	133.33	133.33	133.33	133.33
DTR	42.33	27.77	28.33	29.43	27.77	26.67	26.67	26.67	26.67	26.67
MFTR	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67	26.67
DFTR	26.67	26.67	26.67	26.67	26.67	30.00	34.33	35.67	38.33	38.33

Vit C : Vitamin C **AG** : Gallic acid
D and M tinctures obtained respectively by decoction and maceration.

Reference antioxidants EC_{50} values are smaller than the tested alcoholic tinctures. Quercetin has a more pronounced antioxidant activity (EC_{50} = 0.033) than vitamin C (EC_{50} = 12.23), and gallic acid (EC_{50} = 12.77). This finding was explained by the nature of the intrinsic reactivity seat of each compound as well as the number, the position and the nature of the OH groups.[16,17] Among the tested, only tinctures MF, DTR, MFTR and DFTR have EC_{50} close to those of the reference antioxidants.

CONCLUSION

The results obtained in the term of the present investigation, clearly highlight a notable anti-radical behavior of the alcoholic tinctures analyzed against the stable radical DPPH, giving evidence on the rebound, that *H. rotundifolia* has an undeniable antioxidant virtues. This helps to give a rational explanation of its use in native therapy in Côte d'Ivoire. Hence the need for further characterization of its phytochemical phenolic antioxidants.

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Conflict of Interest: None Declared.

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