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Phytochemical investigation and TLC screening for antioxidant activity of 24 plant species consumed by the Eastern Lowland Gorillas (Gorilla beringei ssp. graueri: Hominidae, Primates) endemic to Democratic Republic of the Congo

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ABSTRACT

Humans and great apes (bonobos, chimpanzees, gorillas, and orangutans) share a common gut anatomy. Although, some diseases that cause countless deaths in humans are ineffective or have minor non disturbing effects in apes. Because of their phylogenetic closeness and common neural pathways of chemosensory perception, humans and great apes, when displaying symptoms of illness could alter their foraging to ingest non-nutritive chemical as diet (pharmacophagy). The aim of the present study was to evaluate the chemical composition and the radical scavenging activity of 24 plants consumed by *Gorilla beringei ssp. Graueri.* Flavonoids and proanthocyanidins plant contents were evaluated by Aluminium nitrate method and vanillin-HCl assay respectively. Antioxidant activity was carried out by TLC bioautography method using 1,1-diphényl1- 2-picrylhydrazyle radical as model. The results of chemical screening revealed the presence of alkaloids, cardiotonic heterosids, tannins, quinones, flavonoids, terpenoids and steroids. 12 plant species *Begonia meyeri-johannis*, *Blotiella crenata*, *Cyathea manniana*, *Englerina woodfordioides*, *Galiniera saxifraga*, *Mimulopsis excellens*, *Myrica mildbraedii*, *Neoboutonia macrocalyx*, *Piper capense*, *Psychotria palustris*, *Solenostemon thyrsiflorum* and *Triumfetta cordifolia* were found to contain flavonoids concentration higher or to equalizes to 1 mg QE/g extract. These plants displayed antioxidant activity thus justifying the role of animal self-medicative behaviour as source of possible epigenome modulators and may aid in the control of infectious diseases through the consumption of non-nutritive phytochemicals by infected animals. The results suggest that zoopharmacognosy might be a promising and complementary source of nutraceuticals for human health care including Sickle cell Disease; an ischemic disease causes by reactive oxygen species.

Keyword: *Gorilla beringei graueri*, medicinal foods, zoo-pharmacognosy, TLC Bioautography, Virunga National Park, Democratic Republic of the Congo

INTRODUCTION

Recent findings have revealed that ethno-pharmacology plays a key role as source of new drugs [1, 2]. This approach using ethno-botanical surveys can provide useful information as a pre-screen to select plant for experimental studies. However, the ethno-pharmacological approach has some limitations in its application particularly the reluctance of traditional practitioners to disclose their secret and the lack of consensus among healers relating to the use of certain medicinal plants. For this purpose, the alternative strategy uses zoo-pharmacognosy approach for identifying bioactive agents from plants or invertebrates [3]. It is a mean by which animal self-heal. The self-medicative behaviour is well documented in non-human primates' practice. Indeed, because of their phylogenetic closeness and common neural pathways of

chemosensory perception, humans and great apes, when displaying symptoms of illness learn to select some biological resources as medicine [4, 5]. Great apes are a good model for human pathology and physiology. The use of plants in self-medication by the non-human primates was reported to be an advantage in protecting them against diseases [6]. In order to discover new effective molecules against human diseases, some researchers have been studying for several years, the self-medication behaviour in wild great apes in order to identify compounds based plants they use for as medicine [5, 6]. The biosynthesis of such secondary metabolites occurs in plants as a result of selective pressure exerted by microbes, phytophagous invertebrates and vertebrates. These compounds protect plant species from predators and pathogens [7]. Surprisingly, some of such non-nutritional metabolites are the major source of drugs for human health care. This is the case of artemisinin, quinine, taxol, morphine and codeine isolated from medicinal plants [8].

The aim of the present study was to evaluate the phytochemical composition and antioxidant activity of 24 plant species consumed by the Eastern Lowland Gorillas (*Gorilla beringei ssp. graueri*: Hominidae, Primates) endemic to the mountainous forest of eastern Democratic Republic of the Congo.

MATERIALS AND METHODS

Plant samples collection and identification

Specimens of twenty-four plant species included in the diet of Eastern Lowland Gorillas (figure 1) were collected in December 2012 in the "Mont Tshiabirimu" (Virunga National Park) and identified first with the help of the field assistants, and by comparison with already identified herbarium specimens collection at the herbarium of the Faculty of Science (University of Kinshasa) with the help of INERA (Institut National d'Etudes et de Recherches Agronomiques) botanist team, especially Mr. Anthony Kikufi, Mr. Zamena Nsita Jonas, and Mr. Nlandu Lukebakio Boniface. Voucher specimens are on deposit at the same herbarium.The plants were collected in the Lubero territory, located in DR Congo, between 0°30' to 0°34' N and 28°00' to 29°30' E.

Extraction and chemical screening

The dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with methanol (MeOH) (100 mL x 2) for 48 hours. Fractions were filtered and concentrated to dryness under reduced pressure using a rotary evaporator. Chemical screening was done using an established protocol as previously reported [9, 10].

Antioxidant activity

The DPPH free radical (1,1-diphenyl-2- picrylhydrazyl) scavenging assay was carried out by TLC bioautography method as previously reported [11]. The radical scavenging activity of extracts for DPPH free radical was measured on the principle that antioxidants reduce the DPPH radical to a yellow-coloured compound (diphenylpicrylhydrazin) and the extent of the reaction will depend on the hydrogen donating ability of the antioxidant. Plant extracts were spotted on silica gel sheets (Silica gel 60 F254 TLC plates) and developed in AcOEt-CH_3COOH-$HCOOH$-H_2O (100: 11: 11: 27) and butanone-2 / toluène (4: 6 ; v/v). Plates were sprayed with methanolic solution of DPPH radical (0, 2%). Chlorogenic acid, cafeic acid, quecertin and isoquercitin were used as reference controls. The active constituents were detected as yellow smear or spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

Polyphenols quantification

Determination of total proanthocyanidin content

The proanthocyanidin content was determined spectrophotometrically in the extracts by the vanillin-HCl assay as previously described [12]. Briefly, 0.5 mL of plant extract solution (0.1 mg/mL) was mixed with 3 ml of 4% de vanilline–MeOH mixture and 1.5 mL of hydrochloric acid. The mixture was allowed to stand for 15 min and the absorbance was monitored at 500 nm using a GENESYS 10S UV-Vis spectrophotometer. The measurements were done in triplicate. For the cathechin standards, a calibration curve (Pearson's correlation coefficient: $R^2 = 0.999$) was constructed and the level of proanthocyanidin for each sample was expressed as cathechin equivalents (mg CE/g extract). The negative control solution consist of 0,5 mL of methanol instead of plant extract.

Determination of flavonoid content

Total flavonoid content was determined spectrophotometrically in the extracts according to the method described by Rahmat et al. [13]. Briefly, 0.25 mL of methanolic plant extract (1 mg/mL) and quecertin standard solution was mixed with 1.25 mL of distilled water in a tube test, followed by addition of 75 µL of a 5% (w/v) sodium nitrite solution. After 6 min, 150 µL of 10% (w/v) $AlCl_3$ solution was added, and the mixture was made up to 2.5 mL with distilled water and mixed well. The absorbance was monitored at 510 nm using a GENESYS 10S UV-Vis spectrophotometer. The measurements were done in triplicate. The results of samples were expressed as mg of quecertin equivalents of total extractable compounds (mg QE/g extract). The negative control solution consists of 1 mL of methanol instead of plant extract.

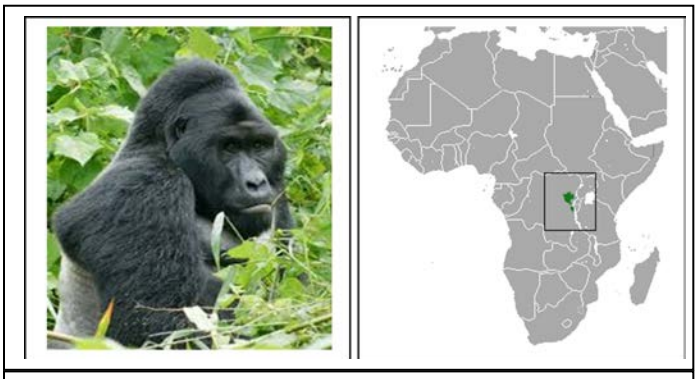

Figure 1: *Gorilla beringei ssp. graueri* (Hominidae, Primates)

RESULTS AND DISCUSSION

Chemical screening

The results of chemical screening 24 plant species are presented in Table 1. These plants are belonging to 19 families and 24 genera. Asteraceae family is the most represented with four species (16.67%) confirming that this family is one of the largest angiosperm families [14].

Table 1: Chemical screening of plant species consumed by *Gorilla beringei graueri* (Matschie, 1914)

It is deduced from the table 1 that on 24 investigated plants, only two (8, 3%) contain alkaloids (*Ilex mitis* and *Xymalos monospora*), six plants (or 25%) contain

	Plant species	Secondary metabolites					
		Alkaloids	Flavonoids	Cardiotonc heterosids	Quinones	Tanins	Terpenoids et steroids
1	*Arundinaria alpina* K Schum.	-	+	-	+	-	+
2	*Basella alba* L.	-	-	-	-	-	+
3	*Begonia meyeri-johannis* Engl.	-	+	-	+	-	+
4	*Blotiella crenata* (Alston) Schelpe	-	+	-	+	-	+
5	*Pteridium centrali-africanum* (Hieron. ex R.E. Fries) Alston	-	+	+	-	+	-
6	*Cyathea manniana* Hook.	-	+	+	+	-	+
7	*Englerina woodfordioides* (Schweinf.) Balle	-	+	+	+	-	+
8	*Galiniera saxifraga* (Hochst.) Bridson	-	+	+	-	+	+
9	*Gynura scandens* O. Hoffm.	-	-	-	-	-	+
10	*Ilex mitis* (L.) Radlk.	+	+	-	-	-	-
11	*Mikania cordata* (Burm.f.) B.L.Rob.	-	-	-	-	-	+
12	*Mikaniopsis* sp.	-	-	-	-	-	+
13	*Mimulopsis excellens* Lindau	-	+	-	+	-	+
14	*Myrica mildbraedii* Engl.	-	+	+	-	+	+
15	*Neoboutonia macrocalyx* Pax	-	+	-	+	-	+
16	*Piper capense* L.f.	-	+	-	+	-	+
17	*Psychotria palustris* E.M.A.Petit	-	+	+	-	+	+
18	*Rapanea melanophloeios*(L) Mez	-	-	-	+	-	+
19	*Rubus kirungensis* Engl.	-	+	-	+	+	+
20	*Solenostemon thyrsiflorum* (Lebrun & L. Touss.) Troupin	-	+	-	+	-	+
21	*Triumfetta cordifolia* A. Rich.	-	+	-	+	+	+
22	*Urera hypselodendron* (Hochst. ex A. Rich.) Wedd.	-	-	-	+	-	+
23	*Vernonia ampla* O. Hoffm.	-	-	-	+	-	+
24	*Xymalos monospora* Baill	+	+	-	-	+	-

cardiotonic heterosids; seven plant species (29,2%) contain the tanins; 14 plants (58,3%) contain quinones; 17 plants (70,8%) contain the flavonoids and 21 plants (87,5%) contain terpenoids and steroids. In terms of the number of secondary metabolites, *Basella alba*, *Gynura scandens*, *Mikania cordata* and *Mikaniopsis sp*. are the plants which contain less compounds, since they contain one chemical group on the six identified ones. Figure 2 shows TLC profiling of plant extracts containing quinones as revealed by NaOH or NH_4OH 10% (colored spots).

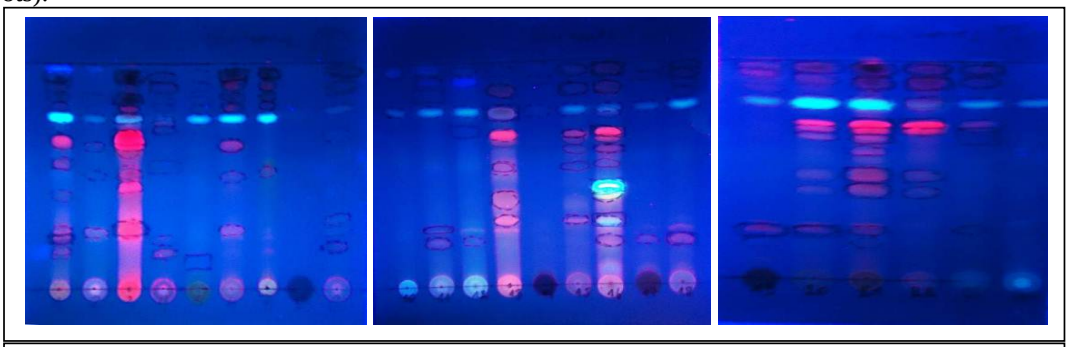

Figure 2 : TLC chromatogram of plant extracts containing quinones (butanone – 2/toluene, 40:60), obsercation under UV lamp at the wavelength of 366 nm.

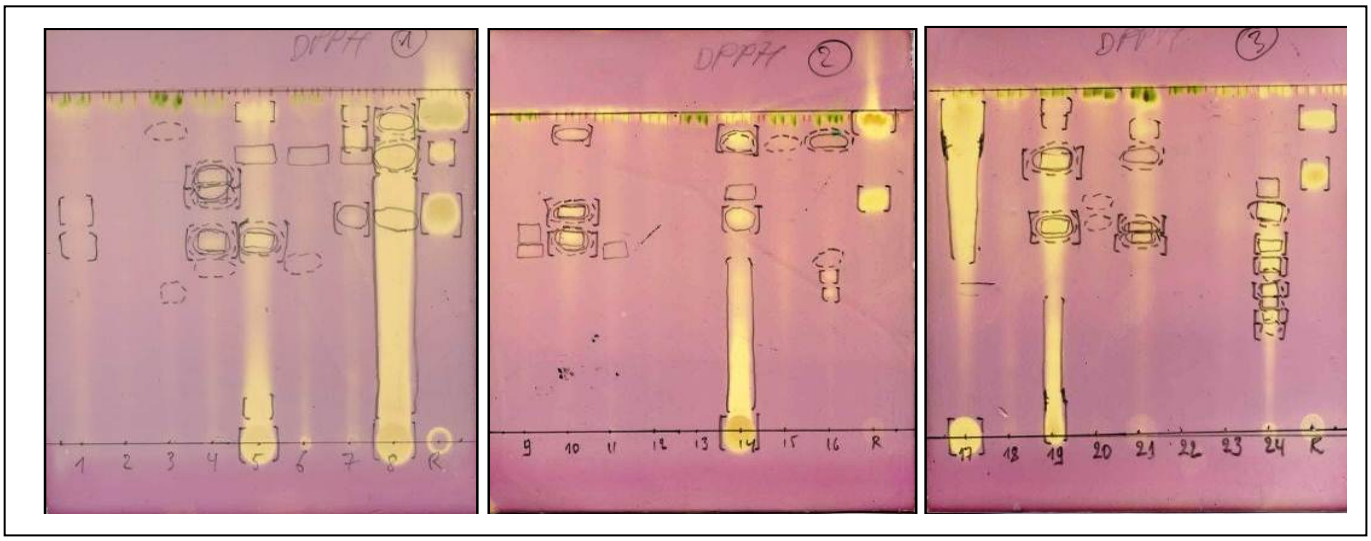

Figure 3: TLC chromatogram of plant extracts displaying radical scavenging activity.

Figure 3 shows TLC bioautography chromatogram profiling of plant extracts containing quinones as revealed by the methanolic solution of DPPH radical (0,2%).

It can deduce from this figure that the plant species *Pteridium centrali-africanum*, *Galiniera saxifraga*, *Myrica mildbraedii*, *Psychotria palustris*, *Rubus kirungensis*, *Triumfetta cordifolia* and *Xymalos monospora* are displayed interesting radical scavenging activity, as revealed by the yellow smear or spots on the bioautobiography TLC chromatograms. Other plants like *Arundinaria alpina*, *Begonia meyeri-johannis*, *Blotiella crenata*, *Gynura scandens*, *Ilex mitis*, *Neoboutonia macrocalyx*, *Piper capense* and *Solenostemon thyrsiflorum* showed a weak anti-oxidant activity.

The table 2 lists the plants species consumed by *Gorilla beringei graueri* in alphabetical order of their scientific names (in italic), followed by their families, their Congolese vernacular names, the used parts and their phenolic content.

N°	Scientific names (Family)	Vernacular names (Nande)	Used parts	Polyphenols content (mg/g DW)	
				Proanthocyanidins	Flavonoids
1	*Arundinaria alpina* K Schum. (Poaceae)	Mulonge	Leaves	0,1815 ± 0,006	0,726 ± 0,003
2	*Basella alba* L. (Basellaceae)	Ndenderu	Whole plant	0,087 ± 0,0001	No evaluated
3	*Begonia meyeri-johannis* Engl. (Begoniaceae)	Virererere	Leaves	0,3 ± 0,0001	1,638 ± 0,014
4	*Blotiella crenata* (Alston) Schelpe (Dennstaedtiaceae)	Muvale	Leaves	0,1 ± 0,0001	2,215 ± 0,0001
5	*Pteridium centrali-africanum* (Hieron. ex R.E. Fries) Alston (Dennstaedtiaceae)	Kasula	Rhizomes	0,574 ± 0,0001	0,734 ± 0,003
6	*Cyathea manniana* Hook. (Cyatheaceae)	Kisembe	Leaves	0,106 ± 0,0001	1,414 ± 0,004
7	*Englerina woodfordioides* (Schweinf.) Balle (Loranthaceae)	Ngatikatika	Leaves	0,127 ± 0,002	1,087 ± 0,002
8	*Galiniera saxifraga* (Hochst.) Bridson (Rubiaceae)	Mulyangote	Stem bark	1,3655 ± 0,0006	0,69 ± 0,002
9	*Gynura scandens* O. Hoffm. (Asteraceae)	Kirimyamuliro	Whole plant	0,11 ± 0,0001	No evaluated
10	*Ilex mitis* (L.) Radlk. (Aquifoliaceae)	Mwise	Stem bark	0,052 ± 0,0001	0,2975 ± 0,0006
11	*Mikania cordata* (Burm.f.) B.L.Rob. (Asteraceae)	Mukohya	Stem bark	0,08 ± 0,0001	No evaluated
12	*Mikaniopsis* sp. (Asteraceae)	Muhururu	Stem bark	0,101 ± 0,0001	No evaluated
13	*Mimulopsis excellens* Lindau (Acanthaceae)	Mughunda	Leaves	0,121 ± 0,0001	2,248 ± 0,0001
14	*Myrica mildbraedii* Engl. (Myricaceae)	Munzikili	Stem bark	1,4575 ± 0,0016	0,636 ± 0,002
15	*Neoboutonia macrocalyx* Pax (Euphorbiaceae)	Vyona	Whole plant	0,124 ± 0,002	2,011 ± 0,003
16	*Piper capense* L.f. (Piperaceae)	Matumbitumbi	Stem bark	0,142 ± 0,0001	2,3245 ± 0,0026
17	*Psychotria palustris* E.M.A.Petit (Rubiaceae)	Mutahitsya	Stem bark	0,3135 ± 0,0006	1,0925 ± 0,0027
18	*Rapanea melanophloeios* (L) Mez (Primulaceae)	Mungokwe	Stem bark	0,112 ± 0,0001	No evaluated
19	*Rubus kirungensis Engl. (*Rosaceae)	Mahwa	Leaves + Stem	0,166 ± 0,003	2,203 ± 0,004
20	*Solenostemon thyrsiflorum* (Lebrun & L. Touss.) Troupin (Lamiaceae)	Viryanzweve	Leaves	0,187 ± 0,0001	2,2835 ± 0,0006

21	*Triumfetta cordifolia* A. Rich. (Malvaceae)	Kitembekali	Leaves	0,3225 ± 0,0006	2,731 ± 0 ,025
22	*Urera hypselodendron* (Hochst. ex A. Rich.) Wedd. (Urticaceae)	Rise	Leaves	0,099 ± 0,0001	No evaluated
23	*Vernonia ampla* O. Hoffm. (Asteraceae)	Mbatule	Stem bark	0,128 ± 0,0001	No evaluated
24	*Xymalos monospora* Baill (Monimaceae)	Kitinde	Stem bark	0,2305 ± 0,0006	0,605 ± 0,004

It is deduced from the table 2 that the plants *Galiniera saxifraga* (1, 3655 ± 0, 0006 mg CE/g extract) and *Myrica mildbraedii* (1, 4575 ± 0, 0016 CE mg/g extract) displayed high amount of proanthocyanidins, but no significant difference were observed between the two plants (P>0.05). Between the lowest and the highest values, the difference was statistically significant (P<0.05); the plant *Urera hypselodendron* having the lowest proanthocyanidins concentration (0,099 ± 0, 0001 CE mg/g extract) while *Myrica mildbraedii revealed t*he highest concentration of proanthocyanidins (1, 4575 ± 0, 0016 CE mg/g extract). According to flavonoids content, the plants *Begonia meyeri-johannis* (1,638 ± 0,014 mg QE/g extracr), *Blotiella crenata* (2,215 ± 0,0001 mg QE/g extract), *Cyathea manniana* (1,414 ± 0,004 mg QE/g extract), *Englerina woodfordioides* (1,087 ± 0,002 mg QE/g extract), *Mimulopsis excellens* (2,248 ± 0,0001 mg QE/g extract), *Neoboutonia macrocalyx* (2,011 ± 0,003 mg QE/g extract), *Piper capense* (2,3245 ± 0,0026 mg QE/g extract), *Psychotria palustris* (1,0925 ± 0,0027 mg QE/g extract), *Rubus kirungensis* (2,203 ± 0,004 mg QE/g extract), *Solenostemon thyrsiflorum* (2,2835 ± 0,0006 mg QE/g extract) and *Triumfetta cordifolia* (2,731 ± 0,025 mg QE/g extract) were found to be rich in flavonoïds. Between the lowest (*Ilex mitis:* 0, 2975 ± 0, 0006 mg QE/g extract) and the highest flavonoids values (*Triumfetta cordifolia:* 2,731 ± 0,025 mg QE/g extract), the difference is also statistically significant (P<0.05). From the table 2, it can also deduce that leaves are rich in flavonoids followed by the barks, while the proanthocyanidins were well represented in *Galiniera saxifraga (*1, 3655 ± 0, 0006 mg CE/g extract) and *Myrica mildbraedii (*1, 4575 ± 0, 0016 mg CE/g extract).

Increasing evidence accumulated over the last decade indicates that reactive oxygen species (ROS) play a key role in the pathophysiology of various ailments including parasitic, chronic and neurodegenerative diseases [15]. The results outlined in this paper, revealed the scavenging effects of some plant species consumed by *Gorilla beringei graueri* indicating that such plant species could protect them from these diseases. Indeed, great apes constitute a reservoir for human pathogens and can serve as sentinels for surveillance of emerging pathogens by providing models for basic research [16]. Natural products were reported to interact with the immune system to either up-regulated or down-regulated specific aspects of the host response by modifying the immune system to enhance the ability of organism to resist invasion by infectious pathogens [17]. It could be hypothesized that the animal self-medicative behavior may aid in the control of infectious diseases through the consumption of non-nutritive phytochemicals by infected animals [18]. So, infectious diseases such as malaria does not seem cause any harm or illness to the great apes like besides the case for the sickle cell disease trait [4, 19-21].

Recent findings indicate that phenolic antioxidants such as flavonoids and proanthocyanidins function as potent modulators of the mammalian epigenome-regulated gene expression through regulation of DNA methylation, histone acetylation, and histone deacetylation in human experimental models. Naturally occurring dietary polyphenols can modulate signaling pathways mediated via NF-κB and MAP kinase, and up-regulate glutathione biosynthesis genes through activation of Nrf2. Polyphenols also down-regulate the expression of pro-inflammatory mediators, matrix metalloproteinases and adhesion molecules by inhibiting histone acetyltransferase activity and activating histone deacetylases [22,23]. It is thus possible that the consumption of antioxidant phenolics such as flavonoids and proanthocyanidins by the great apes especially *Gorilla beringei graueri* can modulate their epigenome in order to protect them from neurodegenerative and/or infectious diseases.

CONCLUSION

The present study evaluated the phytochemical composition and antioxidant activity of 24 plant species consumed by the Eastern Lowland Gorillas. The extracts obtained from some of these plants displayed antioxidant activity. This activity could be due to phenolic compounds such as flavonoids and proanthocyanidins. The ability of extracts from plants consumed by the great apes to display antioxidant properties could partially justify the role of self-medicative behaviour as source of epigenome modulators. These results suggest that zoopharmacognosy might be a promising and complementary source of nutraceuticals for human health care including Sickle cell Disease, an ischemic disease causes by reactive oxygen species.

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