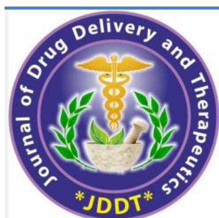

Available online on 15.11.2021 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Copyright © 2021 The Author(s): This is an open-access article distributed under the terms of the CC BY-NC 4.0 which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use provided the original author and source are credited

Open Access Full Text Article

Research Article

Polyphenols contents and antioxidant potential of *Nauclea latifolia* Smith (Rubiaceae) acetonic fractions from Burkina Faso

Konate Almamy [1*], Meda Roland Nâg-Tiéro [2,3], Dicko Amadou [1], Kam Eric Sami [2], Koama Benjamin Kouliga [2,3,4], Belem Hadidjatou [2], Kabore Adama [1], Traore Amadou [1] And Tamboura Hamidou Hamadou [1]

[1] *Laboratoire de Biologie et Santé Animales, Institut de l'Environnement et de recherches agricoles (INERA), 04 BP 8645 Ouagadougou 04, Burkina Faso.*

[2] *Laboratoire De Recherche Et d'Enseignement En Santé Et Biotechnologies Animales, Unité De Formation Et De Recherche En Sciences Et Techniques, Université Nazi Boni, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.*

[3] *Unité de Formation et de Recherche en Sciences et Techniques, Université Nazi Boni, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso.*

[4] *Laboratoire De Médicine Et Pharmacopée Traditionnelle, Institut De Recherche En Sciences De La Santé (IRSS), 01 BP 2779 Bobo-Dioulasso, Direction Bobo-Dioulasso, Burkina Faso.*

Article Info:

Article History:

Received 20 September 2021
Reviewed 28 October 2021
Accepted 03 November 2021
Published 15 November 2021

Cite this article as:

Konate A, Meda RN, Dicko A, Kam ES, Koama BK, Belem H, Kabore A, Traore A, Tamboura HH, Polyphenols contents and antioxidant potential of *Nauclea latifolia* Smith (Rubiaceae) acetonic fractions from Burkina Faso, Journal of Drug Delivery and Therapeutics. 2021; 11(6):101-107

DOI: http://dx.doi.org/10.22270/jddt.v11i6.5069

*Address for Correspondence:

Almamy KONATE, Laboratoire de Biologie et Santé Animales, Institut de l'Environnement et de recherches agricoles (INERA), 04 BP 8645 Ouagadougou 04, Burkina Faso.

ORCID ID: *https://orcid.org/0000-0002-9511-6668*

Abstract

Scientific information on antioxidant properties and phenolic content of *Nauclea latifolia* used in ethnoveterinary medicine in Burkina Faso are limited. Therefore, the quantification of the antioxidant activity of different parts of this specie remains an interesting and useful task, particularly for finding new sources for natural antioxidants. The aim of this study was to evaluate the antioxidant activity and total polyphenols of *Nauclea latifolia* Smith (Rubiaceae) acetonic fractions from Burkina Faso. *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol fractions of leaves, barks and root barks were tested for their antioxidant activities using DPPH, ABTS and FRAP methods. Folin-Ciocalteu and $AlCl_3$ reagents were used to quantify the polyphenols. *n*-butanol fraction of barks (58.16 ± 0.76 mg GAE/100 mg), dichloromethane fraction of barks (51.13 ± 0.99; 26.14 mg GAE/100 mg) have presented the best total phenolic contents while the best total flavonoid contents were found in *n*-butanol fraction of leaves and n-hexane fraction of leaves with 4.85 ± 0.14 mg EQ/100mg and 2.92 ± 0.13 mg EQ/100mg, respectively. It was observed that n-hexane fraction of leaves was scavenge more DPPH free radicals with a value of 1011.98± 17,01 µmol EAA/g. That of *n*-butanol fractions of barks was showed the best ferric reduction power (3056.37 ± 96.66 µmol EAA/g) and the highest ABTS cation radicals scavenging capacity (7031.52 ± 254.98 µmol EAA/g). Nevertheless, this work encourages investigations on Burkina Faso plant species used in the ethnoveterinary medicine as sources of antioxidants.

Keywords: Fraction; Ouagadougou; Barks, Total Polyphenols Contents; Radicals.

INTRODUCTION

Infectious diseases of livestock are a major threat to animal and human health in the world and their effective control is crucial [1,2]. Increases in the emergence or re-emergence of zoonotic infections pose significant additional threats to human health [3,4]. In Burkina Faso, the sanitary situation is characterised by a predominance of many epidemic and endemic diseases, together with a lack of qualified health worlkers. The best way to control of those infectious diseases is a holistic approach, which integrates robust diagnostic practices and vaccination. However, in many development countries, this approach is difficult to apply [5]. Traditional herbal remedies are widely using by many people from Africa for their primary health care and the animal care for several reasons [6,7]. The traditional medicine is considered to be effective methods for curbing infectious diseases, reducing its intensity, shortening its course, or even preventing its recurrences [6,7]. The effectiveness of traditional medicine is mainly due to the choice of products, which optimize the functioning of the immune system and strengthen its reactivity. In addition to offering better and efficacious treatment options, the medicinal plants are also available, easily accessible and with minimal side effects relative to the conventional therapies [8].

Nauclea latifolia Smith (syn. *Sarcocephalus latifolius*, Rubiaceae), is a plant widely used in West Africa for the treatment of several diseases [9-11]. In Burkina Faso, different extracts are used in the management of fevers, malaria, febrile states, dry colic, intestinal parasites, gastroenteritis, dysentery, vomiting, gonorrhea, syphilis, bilharzia and nervous attacks. Phytochemical analysis of extracts of the plant showed the presence of saponins, tannins alkaloids,

flavonoids and phytate [12-15]. More recently, some researchers have discovery from a bio-guided purification of the roots of *Nauclea latifolia*, an active compound natural phytochemical as tramadol. This drug is available as a synthetic analgesic since the 1970s. It is also known to have a strong antibacterial property [16, 18]. Accordingly, it could be interesting to continue the investigating of this plant to ascertain and validate its medicinal value with a view to discovering new leads for better and improved management of infectious disease conditions.

This study was conducted to investigate the antioxidant potential of the fraction n-hexane, dichloromethane, ethyl acetate and n-butanol of different parts of *Nauclea latifolia*. These could be the contributing factors to their health beneficial effects

MATERIAL AND METHODS

Plants Materials

Nauclea latifolia Smith (Rubiaceae) was collected at periphery of Ouagadougou (Burkina Faso) in September 2020. The botanical identification was done by Dr GANABA S. botanist at the National Herbarium of Burkina Faso where voucher specimens were kept. The part of plants collected were washed, dried in the shade at ambient temperature under the fan, crushed, and then sieved to obtain the fines powders.

Preparation of Extracts

The powdered plant samples (25 g) of each part were extracted with 250 ml of acetone (80 %) for 24 hours using an electric mixer. After filtration, acetone was removed using a rotary evaporator at 60°C. The aqueous extract was then fractionated by successive liquid-liquid partitioning with an equal volume of *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. Extracts were stored at 4°C until being used.

Chemicals and reagents-

Chemicals include Folin-Ciocalteu, sodium carbonate, gallic acid, aluminium chloride ($AlCl_3$), sodium acetate, rutin, vanillin, tannic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ABTS (2,2′-azinobis-(3-éthylbenzothiazoline-6-sulfonique)), potassium ferricyanide ((K3Fe(CN)6), trichloroacetic acid, iron III chloride, ascorbic acid, potassium persulfate, quercetin, hexane, ethyl acetate, methanol, n butanol. All chemicals and solvents used were of analytical grade and were purchased from Sigma Aldrich, and local suppliers.

Total Phenolics Content

Total phenolic content of each extract was evaluated according to the spectrophotometric method using the Folin-Ciocalteu reagent described by Meda et *al*., (2010) [19]. A volume of 125 µl (0.1 mg/ml) of each extract was mixed with 625 µl of Folin-Ciocalteu reagent (0.2 N). After an incubation in the dark for 5 minutes, 500 µl of sodium carbonate (7.5% Na_2CO_3) was added to the mixture. After 2 hours' incubation in the dark again, absorbance was read at 760 nm against a standard gallic acid curve (y = 4668e-3 *x-0.034, r^2 = 0.9991). The mean of three readings was used and the results were expressed in mg of gallic acid equivalent per 100 milligrams of dry extract (mg GAE/100mg).

Total Flavonoids Content

The total flavonoids content in the extracts was determined by the aluminum trichloride method described by Meda et *al*., (2010) [19]. To 625 µl (0.1 mg/ml) of each extract solution were added 625 µl of $AlCl_3$ (2%), the whole was mixed and incubated in dark. After 10 minutes, the absorbances were read at 415 nm against a quercetin calibration curve (Y = 1.259e-02 * x; r2 = 0.9990). For each extract the average of 3 measurements was calculated and the amount of flavonoids was expressed in milligrams of quercetin equivalent per 100 milligrams of dry extract (mg QE/100mg).

Antioxidant Activity

DPPH radical method

The antioxidant power was carried out using the DPPH method according to the spectrophotometric method described by Meda et *al*., (2013).[20] In a test tube containing 0.375 ml of the sample (0.1 mg/ml) of each extract, 0.75 ml of the DPPH solution (20 mg /l) was introduced. After 15 minutes' post-incubation of the mixture at room temperature in the dark, the absorbances were immediately read at 517 nm with a spectrophotometer (GENESYS 30). The radical scavenging activity (RSA) of each extract was measured using a reference curve of ascorbic acid. The antioxidant activity was expressed in µmol equivalent ascorbic acid per 1 gram of dry extract (µmol EAA/g).

ABTS radical cation decolorization assay

The ABTS cation radical ($ABTS^{\bullet+}$) scavenging capacity of antioxidants was determined as described by Meda et *al*., (2010). $ABTS^{\bullet+}$ was regenerated by adding an aqueous solution of ABTS (7 mM) to 2.5 mM potassium persulfate solution, and the mixture is kept in the dark at room temperature for 12 hours before use. The mixture solution was then diluted with ethanol and the absorbance was adjusted to 0.700 (± 0.02) at 734 nm using spectrophotometer. 10 µl of each sample was mixed with 990 µl of the $ABTS^{\bullet+}$ solution and incubated in the dark. After 15 minutes the absorbances were measured at 734 nm against a standard curve of ascorbic acid. 3 measures were carried out for each extract and the results were expressed in µmol equivalent ascorbic acid per 1 gram of dry extract (µmol EAA/g).

Iron (III) to iron (II) reduction activity (FRAP)

The FRAP assay was conducted following the method described by Meda et *al*., (2010). Briefly, 0.5 ml of extract (100 µg ml^{-1}) was added to 1.25 ml of phosphate buffer (0.2 M, pH 6.6) and 1.25 ml of potassium ferricyanide solution (1%). The resulting mix was then incubated at 50°C in a water bath. After 30 minutes 1.25 ml of trichloroacetic acid (10%) was added and the mixtures were centrifuged for 10 min at 3000 rpm. The supernatants of solutions (0.625 ml) were added to distilled water (0.625 ml) and to freshly prepared $FeCl_3$ solution (0.125 ml; 0.1%). The absorbances were read at 700 nm against a standard curve of ascorbic acid. The tests were performed in triplicate and expressed in µmol equivalent ascorbic acid per 1 gram of dry extract (µmol EAA/g).

Statistical Analysis

The results were expressed as mean ± standard error of the mean (SEM). 2 way ANOVA multiple comparisons was applied for the statistical analysis, followed by Tukey's multiple comparisons test for post-hoc analysis. Statistical analyses were performed using GraphPad prism 9.12 and a p-value <0.05 was considered to be statistically significant.

RESULTS

Total Polyphenols Contents

As a basis, total phenolics content was measured using the Folin–Ciocalteu reagent in each fraction. The results were

derived from a calibration curve (y = 4668e-3 *x-0.034, r^2 = 0.9991) of gallic acid and expressed in gallic acid equivalents (GAE) per 100 milligrams dry extract weight. The content of phenolics in different fractions ranged from 2.05 to 58.16 mg GAE/100 mg. *n*-butanol fraction of bark, dichloromethane fraction of barks, ethyl acetate fraction of leaves and *n*-butanol fraction of leaves had the greatest phenolics contents (58.16 ± 0,76; 51.13 ± 0.99; 26.14 ± 0.61and 25.92 ± 0.91mg GAE/100 mg, respectively), while the smallest phenolics contents were found in root barks fractions of *n*-hexane fraction, root barks fractions of ethyl acetate fraction and root barks fractions of *n*-hexane fraction (2.05 ± 0.02; 2.21 ± 0.04 and 5.26 ± 0.16 mg GAE/100mg, respectively).

As a basis, total flavonoids content was measured using aluminum trichloride reagent. The results were derived from a calibration curve (Y = 1.259e-02 * x; r2 = 0.9990) of quercetin equivalent and expressed in milligrams of quercetin equivalent per 100 milligrams of dry extract (mg QE/100mg). The total of flavonoids in different fractions ranged from 0.07 ± 0.01 to 4.85 ± 0.14mg QE/100mg. The leaves fractions have presented the greatest flavonoids contents and the smallest flavonoids contents were found in dichloromethane fraction of stem and root barks.

Table 1: Phenolic content (TPC) and flavonoid contain (TFC) in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of roots, stem barks and leaves of *Nauclea latifolia*.

PARTS	SOLVENT	TPC (mgEAG/100mg)	TFC (mgEQ/100mg)
Leaves	*n*-hexane	22.55 ± 0.57	2.92 ± 0.13
	dichloromethane	18.42 ± 0.61	2.91 ± 0.12
	Ethyl acetate	26.14 ± 0.61	2.86 ± 0.22
	n-Butanol	25.92 ± 0.91	4.85 ± 0.14
Stem barks	*n*-hexane	5.26 ± 0.16	----------------
	dichloromethane	51.13 ± 0.99	0.07 ± 0.01
	Ethyl acetate	12.75 ± 0.76	0.39 ± 0.51
	n-Butanol	58.16 ± 0.76	0.68 ± 0.06
Root barks	*n*-hexane	2.05 ± 0.02	0.24 ± 0
	dichloromethane	19.92 ± 0.30	0.36 ± 0.06
	Ethyl acetate	2.21 ± 0.04	0.64 ± 0
	n-Butanol	18.53 ± 0.15	0.32 ± 0

Values are the mean of three replicates (TPC) ±SEM.

In vitro antioxidant activity determination

Trolox Equivalence Antioxidant Capacity in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of Nauclea latifolia.

The results also showed a higher antioxidant content to capture the free radical (ABTS) in the different fractions leaves followed respectivily by the fractions of stem barks and he different fractions of root barks.

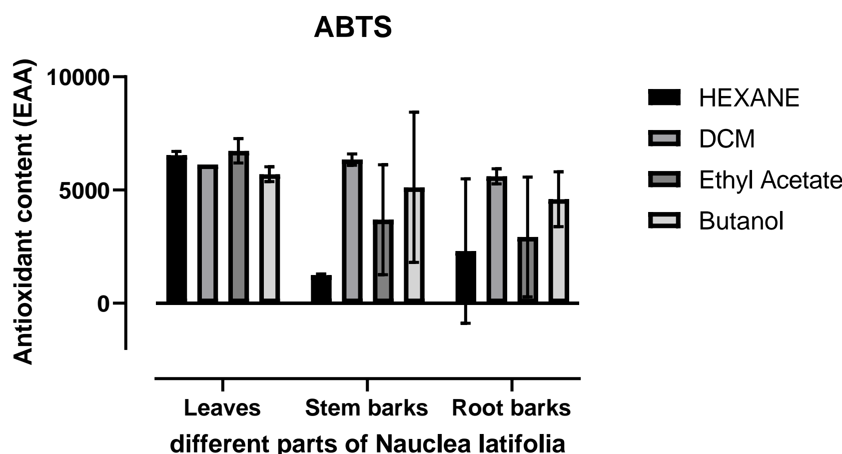

Figure 1: Antioxidant activity by ascorbic acid equivalent antioxidant capacity (ABTS) method in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*. Values are the mean of three replicates (ABTS) ±SEM

Table 2: Tukey's multiple comparisons test of Antioxidant activity by ascorbic acid equivalent antioxidant capacity (ABTS) method in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*

Tukey's multiple comparisons	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value
HEXANE					
Stem barks vs. Leaves	-5298	-5794 to -4802	Yes	****	<0,0001
Root barks vs. Leaves	-4233	-15025 to 6559	No	ns	0,256
Root barks vs. Stem barks	1065	-9770 to 11900	No	ns	0,8443
DCM					
Stem barks vs. Leaves	216	-633 to 1066	No	ns	0,4473
Root barks vs. Leaves	-529	-1662 to 604	No	ns	0,1944
Root barks vs. Stem barks	-745	-1634 to 143	No	ns	0,0827
Ethyl Acetate					
Stem barks vs. Leaves	-3041	-10716 to 4633	No	ns	0,2745
Root barks vs. Leaves	-3799	-12251 to 4653	No	ns	0,2222
Root barks vs. Stem barks	-758	-8167 to 6651	No	ns	0,9301
Butanol					
Stem barks vs. Leaves	-577	-11673 to 10519	No	ns	0,9527
Root barks vs. Leaves	-1106	-4825 to 2614	No	ns	0,423
Root barks vs. Stem barks	-529	-10186 to 9129	No	ns	0,9641

DPPH Radical Scavenging Activity in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of Nauclea latifolia.

In this study, n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of Nauclea latifolia were able to show free radical scavenging abilities (Figure 2). It was observed that the different fractions leaves had higher DPPH activity followed respectively by the fractions of stem barks and he different fractions of root barks.

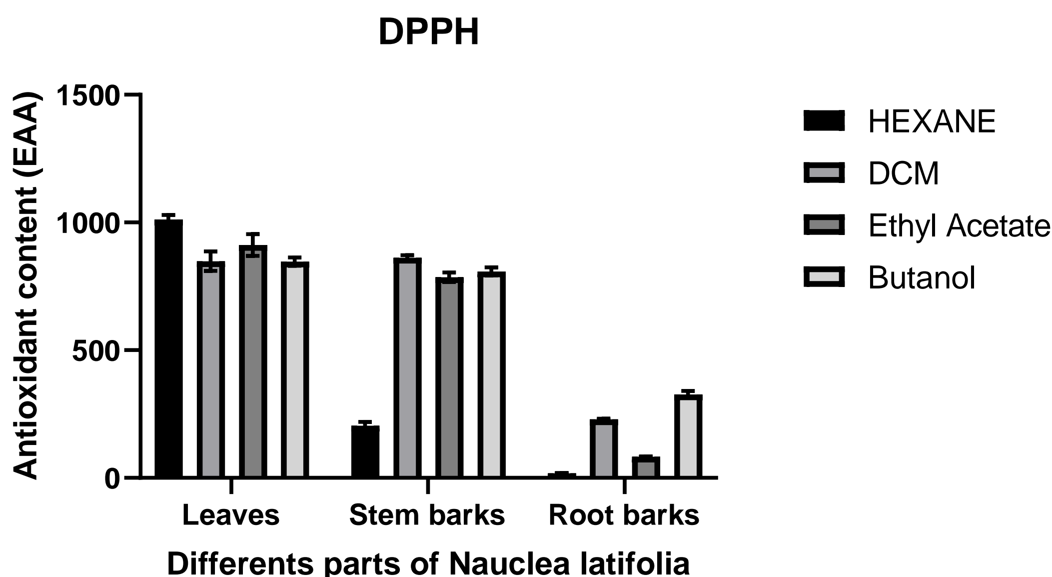

Figure 2: Antioxidant activity by 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*. Values are the mean of three replicates (DPPH) ±SEM

Table 3: Tukey's multiple comparisons test of Antioxidant activity by DPPH radical scavenging capacity method in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*

Tukey's multiple comparisons te	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value
HEXANE					
Stem barks vs. Leaves	-808	-849 to -766	Yes	****	<0,0001
Root barks vs. Leaves	-994	-1036 to -953	Yes	****	<0,0001
Root barks vs. Stem barks	-186	-228 to -145	Yes	****	<0,0001
DCM					
Stem barks vs. Leaves	12,8	-28,6 to 54,2	No	ns	0,7249
Root barks vs. Leaves	-619	-660 to -577	Yes	****	<0,0001
Root barks vs. Stem barks	-632	-673 to -590	Yes	****	<0,0001
Ethyl Acetate					
Stem barks vs. Leaves	-126	-167 to -84,5	Yes	****	<0,0001
Root barks vs. Leaves	-828	-869 to -787	Yes	****	<0,0001
Root barks vs. Stem barks	-702	-743 to -661	Yes	****	<0,0001
Butanol					
Stem barks vs. Leaves	-38,3	-79,7 to 3,10	No	ns	0,0736
Root barks vs. Leaves	-520	-561 to -479	Yes	****	<0,0001
Root barks vs. Stem barks	-482	-523 to -440	Yes	****	<0,0001

Ferric Reducing Antioxidant Power in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of Nauclea latifolia.

The results also showed n-butanol fraction and DCM fraction of the stem barks had higher FRAP abilities to reduce ferric ions, followed by the all fractions of leaves and the ethyl acetate fraction of the stem barks. Root barks fractios showed lower FRAP abilities to reduce ferric ions.

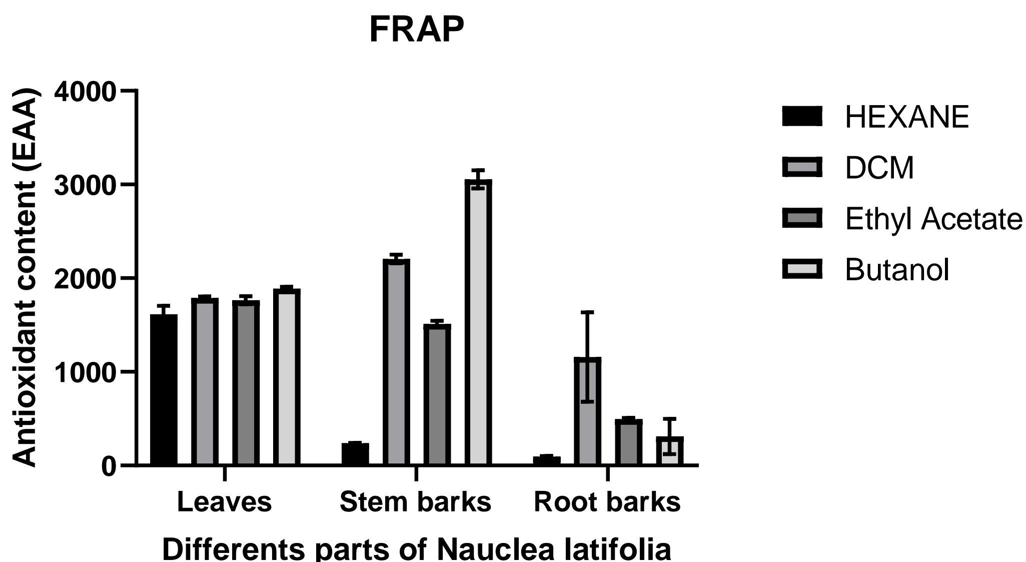

Figure 3: Antioxidant activity by ferric reducing (FRAP) method in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*. Values are the mean of three replicates (FRAP) ±SEM

Table 4: Tukey's multiple comparisons test of Antioxidant activity ferric reducing (FRAP) in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of different parts of *Nauclea latifolia*

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Below threshold?	Summary	Adjusted P Value
HEXANE					
Stem barks vs. Leaves	-1377	-1683 to -1070	Yes	**	0,0018
Root barks vs. Leaves	-1518	-1823 to -1213	Yes	**	0,0011
Root barks vs. Stem barks	-142	-157 to -127	Yes	****	<0,0001
DCM					
Stem barks vs. Leaves	417	284 to 550	Yes	**	0,0031
Root barks vs. Leaves	-631	-2247 to 986	No	ns	0,2574
Root barks vs. Stem barks	-1047	-2643 to 549	No	ns	0,1101
Ethyl Acetate					
Stem barks vs. Leaves	-254	-370 to -137	Yes	**	0,0039
Root barks vs. Leaves	-1269	-1405 to -1133	Yes	****	<0,0001
Root barks vs. Stem barks	-1016	-1109 to -922	Yes	****	<0,0001
Butanol					
Stem barks vs. Leaves	1169	861 to 1478	Yes	**	0,0028
Root barks vs. Leaves	-1577	-2202 to -951	Yes	**	0,0079
Root barks vs. Stem barks	-2746	-3255 to -2237	Yes	***	0,0004

DISCUSSION

Polyphenol contain and antioxidant activity in n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of root barks, stem barks and leaves of *Nauclea latifolia* from Burkina Faso were evaluated. Polyphenols are known as potent antioxidants and contribute to some beneficial properties such as anti-viral and anti-microbial, anti-allergic, anti-inflammatory and immunomodulatory properties [21-26]. The polyphenols can be quantified by various methods, in this study, the total polyphenolic contents of the different fractions part of root barks, stem barks and leaves is determined using the diluted Folin-Ciocalteu reagent [27]. The total phenol and flavonoid content of the different fractions of the plant are presented in Table 1. The n-butanol fraction of root barks, dichloromethane fraction of root barks, ethyl acetate fraction of leaves and n-butanol fraction of leaves had the greatest phenolic contents. This variation could be explaining to various reasons such as solubility and polarity, environmental factors and origin of *N. latifolia samples.* Several analytical methods have been developed to assess the antioxidant properties of the medicinal plant extracts [28].

In this study, the quantification of antioxidant activities of the different fractions have been focused on the DPPH, FRAP and ABTS methods of root bark, stem barks and leaves of *Nauclea latifolia*. The assays of antioxidants by ABTS and DPPH tests are based on the mechanism of the suppression of oxidative stress by the ability to scavenge free radicals. The assays of antioxidants by FRAP method is focused on the measure the ability of antioxidants to reduce ferric (Fe3+) ions. Antioxidants are broadly classified in 4 groups because of the complex nature of the redox-antioxidant system in vivo as: free radical scavengers, inhibitors of free radical formation, cellular and tissue damage repairers, and signaling messengers [29,30]. The results generated from this study demonstrated that different fractions of leaves followed by the fractions of stem barks and the different fractions of root barks respectively possessed good free radical scavenging activity.[29]. The inhibition of free radical formation could protect against oxidative damage by suppressing the formation of active ROS/RNS [29,30]. These fractions could act as antioxidants, and serving possibility as scavengers or free radical inhibitors. Furthermore, antioxidants might have contributed to the different medicinal properties of this plant as reported [31].

Those results could be explained by the fact the antioxidant activity is greatly determined by the chemical structure and electron donation/reception capability of those polyphenols. Moreover, polyphenols due to the presence of a hydroxyl group are more soluble in polar organic solvents; therefore the extraction procedures and solvents could explain our results.

CONCLUSION

In this study, the assessment of antioxidant activity has indicated that all parts of this plant had phenolic and flavonoid contents to varying degrees. This plant could be a source of natural antioxidants against several animal's diseases. The quantification of antioxidant activity could serve as a indicators for the use of these parts of *Nauclea latifolia* against many human and animals diseases. Further works are necessary to better understand their ability to control animals infectious diseases.

ACKNOWLEDGMENT

The authors acknowledge the support of Feed the future through the ENHANCE project which provided the PhD grant and financed this work.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Jones BA, Grace D, Kock R, Alonso S, Rushton J, Said MY, Pfeiffer DU et al, Zoonosis emergence linked to agricultural intensification and environmental change. Proc Natl Acad Sci 2013; 110(21):8399-8404 . https://doi.org/10.1073/pnas.1208059110

2. Karesh WB et al, Ecology of zoonoses: natural and unnatural histories. Lancet 2012; 380:1936-1945 . https://doi.org/10.1016/S0140-6736(12)61678-X

3. Food and Agriculture Organization of the United Nations (FAO) (2006). - Livestock's long shadow: environmental issues and options. FAO, Rome, Italy, 416 pp. Available at: www.fao.org/3/a0701e/a0701e.pdf (accessed on 31 August 2020).

4. Allen T., Murray K.A., Zambrana-Torrelio C., Morse S.S., Rondinini C., Di Marco M., Breit N., Olival K.J. & Daszak P. (2017). - Global hotspots and correlates of emerging zoonotic diseases. Nat. Comm., 8, Article No. 1124. doi:10.1038/s41467-017-00923-8 https://doi.org/10.1038/s41467-017-00923-8

5. Jaffar, S., Amuron, B., Foster, S., et al. Rates of virological failure in patients treated in a home-based versus a facility-based HIV-care model in Jinja, southeast Uganda: a cluster-randomised equivalence trial. Lancet, 2009; 374:2080-9. https://doi.org/10.1016/S0140-6736(09)61674-3

6. Mhame PC, Busia K, Kasilo MJK. Clinical practice of African traditional medicine. African Health Monitor. 2010. Issue 13. Available at: www.aho.afro.int/en/ahm/issue/3 [Accessed: September 23, 2021]

7. World Health Organization (2002). WHO Traditional Medicine Strategy 2002-2005. Geneva: WHO Press;

8. Rafal S. (2014). Mon guide des médecines douces pour une santé au quotidien. Marabout Référence,

9. Kokwaro JO. (1976). Medicinal plants of East Africa. East African Literature Bureau, Nairobi.

10. Akabue P, Mittal GC. Clinical evaluation of a traditional herbal practice in Nigeria: a preliminary report. Journal of Ethnopharmacology, 1982; 6:355-359. https://doi.org/10.1016/0378-8741(82)90056-3

11. Muazu J, Kaita AH. A review of traditional plants used in the treatment of epilepsy amongst the Hausa/Fulani tribes of Northern Nigeria. African Journal of Traditional, Complementary, and Alternative Medicines, 2008; 5:387-390. https://doi.org/10.4314/ajtcam.v5i4.31294

12. M. F. Mahomoodally (2013). "Traditional medicines in Africa: an appraisal of ten potent African medicinal plants," Evidence- https://doi.org/10.1155/2013/617459

Based Complementary and Alternative Medicine.

13. Fernandez de la Pradilla C. (1982). Plantes Medicinales contre les hepatitis: 51 especes tropicales. Ed. Librairie Jeunesse d'Afrique, Ouagadougou, 61.

14. Fernandez de la Pradilla C. (1982). Plantes Medicinales vendue sur les marches de Ouagadougou. Ed. Librairie Jeunesse d'Afrique, Ouagadougou, 22.

15. Maduabunyi W. Anti-hepatoxic and trypanocidal activities of the ethanolic extract of Nauclea latifolia root bark. Journal of Herbs Spices and Medicinal Plants, 1995; 3:23-53. https://doi.org/10.1300/J044v03n02_04

16. A. Benabbou, M. B. Khaled, and A. S. Alchalabi, "Evaluation of the Efficiency of Combined and Separated Antioxidant Supplementation of Vitamin C and E on Semen Parameters in Strepto-zotocin-Induced Diabetic Male Wistar Rats," South Asian Journal of Experimental Biology, vol. 2018; 7(4):166-72. https://doi.org/10.38150/sajeb.7(4).p166-172

17. Nacoulma-Ouédraogoo. G., (1996). Plantes médicinaleset pratiques médicales traditionnelles au Burkina Faso : casdu plateau central. Thèse de doctorat d'État, Faculté dessciences et techniques, Université de Ouagadougou, Bur-kina Faso, tome 1, 320 p., tome 2, 285 p

18. A. D. T. Goji, A. Mohammed, Y. Tanko, I. Ezekiel, A. O. Okpanchi, and A. U. U. Dikko, (2010). "A study of the anti-inflammatory and analgesic activities of aqueous extract of Nauclea latifolia leaves in Rodent," Asian Journal of Medical Sciences, vol. 2, no. 6, pp. 244-247.

19. Meda, N-T.R., (2010).. Phytochemistry study and biological activities of galls and the leaves of Balanites aegyptiaca (L.) Del.(Balanitaceae) used in traditional medicine in Burkina Faso. Ph.D. Thesis, University of Ouagadougou. 154p.

20. Meda N.T.R, Bangou M.J, Bakasso S. Millogo-Rasolodimby J. and O.G. Nacoulma., Antioxidant activity of phenolic and flavonoid fractions of Cleome gynandra and Maerua angolensis of Burkina Faso. J App Pharm Sci. 2013; 3(02):036-042. https://doi.org/10.7324/JAPS.2013.30207

21. A. D. T. Goji, A. Mohammed, Y. Tanko, I. Ezekiel, A. O. Okpanchi, and A. U. U. Dikko, "A study of the anti-inflammatory and analgesic activities of aqueous extract of Nauclea latifolia leaves in Rodent," Asian Journal of Medical Sciences, 2010; 2(6):244-247

22. Haudecoeura R., Peuchmaura M., Pérèsa B., Romec M., Taïwee G. S., Boumendjela A., and Bou-cherlea, B. Traditional uses, phytochemistry and pharmacological properties of African Nauclea species: A review. Journal of Ethnopharmacology 2018 ; 212:106-136. https://doi.org/10.1016/j.jep.2017.10.011

23. Miliauskas, G.; Venskutonis, P.R.; van Beek, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004; 85:231-237. https://doi.org/10.1016/j.foodchem.2003.05.007

24. Dunnill C, Patton T, Brennan J et al. "Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process," International Wound Journal, vol. 2015; 1(8):1-8,. https://doi.org/10.1111/iwj.12557

25. Han X. and Loa T. Dietary polyphenols and their biological significance.International Journal of Molecular Science,; 2007; 8:950-988 https://doi.org/10.3390/i8090950

26. Pandey K.B. and Rizvi S.I. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity, 2009; 2:270-278. https://doi.org/10.4161/oxim.2.5.9498

27. Singleton VL, Orthofer R, Lamuela-Raventós RS. "Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent," Methods in Enzymology, 1998; 299:152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

28. Oyedeji-Amusa MO, Ashafa AOT, Medicinal properties of whole fruit extracts of Nauclea latifolia Smith.: Antimicrobial, antioxidant and hypoglycemic assessments; South African Journal of Botany 2019; 121:105-113 https://doi.org/10.1016/j.sajb.2018.11.001

29. Niki E,"Assessmentofantioxidantcapacityinvitroandinvivo,"Free Radical Biology &Medicine, 2010; 49(4):503-515. https://doi.org/10.1016/j.freeradbiomed.2010.04.016

30. Kellett ME, Greenspan P, Pegg RB, "Modification of the cellular antioxidant activity (CAA) assay to study phenolic antioxidants in a Caco-2 cell line," Food Chemistry, 2018; 244:359-363. https://doi.org/10.1016/j.foodchem.2017.10.035

31. Kumar A, Kaur R, Thind T.S., Aora R., Kaur P. Aora S. Relative evaluation of two euphorbiaceae plants for their hydrogen donating and hydroxyl radical scavenging activities. Journal of Chemical and Pharmaceutical Research, 2015; 7(11):86-90.