# Phenolic content and DPPH scavenging activity of *Carpolobia lutea* and *Anchomanes difformis*

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**Abstract**

New antioxidants molecules especially from natural sources are of great interest today. The leaves and rhizomes of *Anchomanes difformis* and fruits of *C. lutea* were screened for the presence of chemicals constituents. The free radical scavenging activity using DPPH and total phenolic contents using Folin-Ciocalteu reagent were carried out on the ethanolic extracts of *A. Difformis* and *C. Lutea*. Both extracts of *A. difformis* and *C. lutea* were found to contain alkaloids and reductive substances. Leaves and rhizomes of *A. difformis* and fruits of *C. lutea* were found to contain (10.45±0.07), (21.13±1.11) and (4.47±0.27) mg of catechin/g of sample extract respectively. Fruits of *C. lutea* with $IC_{50}$ 2.02 mg/mL have showed high DPPH scavenging activity than leaves and rhizomes of *Anchomanes difformis* with $IC_{50}$ 6.49 mg/mL and 3.15 mg/mL respectively. *A. diformis* and *C. lutea* might be potential sources of bioactive phytochemicals. Further studies need to be done to isolate and to identify these chemical constituents.

Key Words: *Anchomanes difformis*, *Carpolobia lutea*, oxidative stress

## 1. Introduction

The body produces daily oxygen derived free radicals or Reactive Oxygen Species (ROS), products of oxido-reduction reactions with one or more unpaired electrons, which makes them extremely reactive [1]. The instability of these free radicals or reactive oxygen species ($O_2$•, HO•, RO•, ROO•, etc) gives them the ability to pull one or more electrons to the neighbouring molecules. In doing so, they may cause direct damage to biological molecules such as DNA, proteins, lipids or carbohydrates as well as cellular injuries [2]. These chemical attacks resulting from an imbalance between the generation of oxidizing molecules and their neutralization by natural or ingested antioxidants are known under the general term "oxidative stress". Oxidative stress is known to be the main cause of many diseases and infections of their complications such as cancer, diabetes, asthma and neurodegenerative diseases [3, 4].

In recent years, the search for new antioxidants molecules especially from natural sources, mainly plants, became of a capital scientific interest. Several plants have been reported to have good antioxidant activity on one hand and synthetic antioxidants currently been used were found toxic for the consumer and without action on some free radicals like hydrogen peroxide, nitroxide and peroxynitrite ($H_2O_2$, NO•, OONO•) on the other hand [2, 5, 6,7].

Natural antioxidants are present in all parts of higher plants and are in general phenolic and polyphenolic compounds [8]. Epidemiological evidence shows that people who consume large amounts of fruits and vegetables have reduced risk of contracting Alzheimer disease and many types of cancer [9, 10].

We focus on the ethanolic extracts of two edible plants: *Anchomanes difformis* (leaves and rhizomes) and *Carpolobia lutea* (fruits) for phytochemicals, total phenolics content and antioxidant potentials. *Anchomanes difformis*, Araceae is an herbaceous plant growing in moist and shady places of tropical African forests. The powdered root mixed with palm oil is used to treat respiratory diseases, diabetes, oral and anal lesions, tuberculosis and malaria [11, 12]. *Carpolobia lutea*, Polygalaceae is a small tree growing in tropical rain forest. The decoction of the leaves is used to treat leprosy, venereal diseases, various forms of mental disorders like dementia and in the management of impotence [13, 14]. Extracts of *A. difformis* and *C. Lutea* has been reported to exhibit anti-trypanosomal activity [15].

## 2. Materials and methods

Chemicals and reagents: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), anhydrous sodium carbonate, ascorbic acid, cathechin and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich Co., Germany. All other chemicals and reagents used were of analytical grade.

Plant material: *Anchomanes difformis* and *Carpolobia lutea* was collected in the month of May, 2014 at Boumnyebel, Nyong-et-Kelle Division, Cameroon and identified by Mr Kenfack, a Botanist of the Cameroon National Herbarium where a specimen of each plant was deposited.

Extraction: The leaves and rhizomes of *A. difformis* and fruits of *C. lutea* were air dried and pulverized to powder to give 23 g, 10.5 g and 14.2 g of powder respectively. The above mentioned powders were extracted with 100 mL ethanol at room temperature during 3 days. The extracts were filtered using Whatman filter paper no. 2, and concentrated with a rotary evaporator to afford 1.8 g, 4.3 g and 8.6 g of rhizomes and leaves of *A. difformis* and fruits of *C. lutea* extracts respectively.

Phytochemical screening of extracts: Phytochemical screening was carried out on the three extracts to detect the presence of secondary metabolites such as alkaloids, flavonoids and tannins according to procedures described by Trease and Evans (1989) [16].

Total phenolics content using Folin-Ciocalteu reagent: Total phenolic contents were assessed using a modified version of the method described by Demiray *et al*. 2009 [17] with catechin as standard. The Folin reagent (diluted 1:10 in water, 1.5 µL) and aqueous $Na_2CO_3$ (75 g/L, 1.2 µL) were successively added to the herb extract (300 µL). Calibration curve was prepared by mixing methanol solution of catechin (300 µL; 0.125-2 mg/mL) with 1.5 µL of Folin-Ciocalteu reagent (diluted tenfold) and sodium carbonate (1.2 µL, 0.7 M). Absorbance values were measured after 30 min at 765 nm using a UV-VIS spectrophotometer and the standard curve was plotted. All determinations were carried out in triplicate. The total phenolics content in the extracts were expressed in catechin equivalent (CE) and were calculated by the following formula: T = CxV/M; where T = total phenolic contents, milligram per gram of sample extract, in CE; C = the concentration of catechin established from the calibration curve, mg/mL; V = the volume of extract, milliliter; M = the weight of sample extract (g)

Measurement of free radical scavenging activity using DPPH method: The free radical scavenging activity of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were measured spectrophotometrically following the modified standard method described by Blois (1958) [18]. Different concentrations (1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0125625 mg/mL) of methanolic solution of extracts and standard were prepared. A volume of 100 µL of each preparation was mixed with 1.9 mL of the methanol solution of DPPH (0.1 mM). An equal amount of methanol and DPPH without sample was served as a control. After 20 min of reaction at room temperature in the dark, the absorbance was measured at 517 nm against methanol as blank. The free radical scavenging activity percentage was calculated using the formula: % scavenging activity = 100x(Ac-As)/Ac where Ac = absorbance of control and As = absorbance of sample. Ascorbic acid was used as standard. The values obtained were plotted on a graph of % change in absorbance versus concentration of samples and $IC_{50}$ value of each extract was calculated.

## 3. Results and discussion

Medicinal plants has been used as remedy since century and has been found to be sources of antibiotic substances like glycosides, saponins, terpenoids, alkaloids, flavonoids, etc [19]. Phytochemical screening confirmed the presence of various classes of secondary metabolites in *A. difformis* and *C. lutea*. Results are showed in Table 1. Both leaves and rhizomes of *A. difformis* contain saponins, flavonoids, alkaloids, anthraquinones and reducing substances. Leaves of *A. difformis* do not contain tannins, phenols, terpenoids and coumarins while the rhizomes contains and these later do not contain steroids and anthracenic glycosides compared to leaves. *C. lutea* fruits contain tannins, phenols, alkaloids, coumarins and anthracenic glycosides and do not contains phenols. Previous phytochemical study revealed the presence of alkaloids, saponins and tannins in both leaves and rhizomes of *A. difformis* harvested in Nigeria [12]. The total phenolic content of *A. difformis* leaves and rhizome and *C. lutea* fruits ethanolic extracts expressed in terms of CE were found to be (10.45±0.07), (21.13±1.11) and (4.47±0.27) mg of catechin/g of sample extract respectively. The results were obtained using the linear equation A = 5.0566X + 0.01 $R^2$ = 0.9746 based on the calibration curve of catechin where A is absorbance and X the amount of catechin in mg. The free radical scavenging activity of different concentrations of each extract were recorded and compared to that of ascorbic acid. Fig. 1 shows the results expressed as inhibition percentage. Minimum inhibition concentration ($IC_{50}$) of each extract was calculated and was found to be equalled to 0.06 mg/mL, 6.49 mg/mL, 3.15mg/mL and 2.02 mg/mL for Ascobic acid, *A. difformis* leaves, *A. difformis* rhizomes and *C. lutea* fruits respectively. Plant extracts has very low activity compared to standard Ascorbic acid. The activity ratio of extracts to standard is about 100, 50 and 30 for leaves and rhizomes of *A. difformis* and fruit *C. lutea* respectively.

Secondary metabolites contained in plants are responsible of their biological activities and constitute an incredible justification for their use in folk medicine. Phenolic compounds particularly are good radical scavengers. They have with their multiple –OH (hydroxyl) function the ability to trap free radicals like DPPH by releasing their hydrogen atom leading to a decrease in its absorbance which can be noticed by a discoloration of its purple colour [20]. Rhizomes of *A. difformis* were found to contain more phenols than its leaves and *C.*

*lutea* fruits. However fruits of *C. lutea* with $IC_{50}$ 2.02 mg/mL have a free radicals inhibitory power higher than those of rhizomes and leaves of *A. differmis* $IC_{50}$ 6.49 mg / mL and 3.15 mg/mL respectively. This result could be explained by the fact that fruits from plants are generally rich in flavonoids which has also been seen to have free radical scavenging capacity [21]. Antioxidant activity of fruits of *C. lutea* is evaluated here for the first time. Results showed that the ethanolic extracts of *C. lutea* have a moderate free radical scavenging activity. This moderate activity may result from various interactions between the different chemical components of the plants.

## CONCLUSION

Our results offer a scientific basis for the use of *A. differmis* and *C. lutea* in traditional medicine to treat related oxidative stress diseases like diabetes, tuberculosis and mental diseases. *A. differmis* and *C. lutea* are rich sources of phytochemicals. *C. lutea* fruits showed a high DPPH free radical scavenging activity compared to *A. differmis* rhizome and leaves. Isolation and characterisation of their phytochemicals could lead to the identification of compound(s) with antioxidant or other therapeutic activity and this might be a subject to examine for further studies.

## REFERENCES


[1] G. Vansant, Free radicals and antioxidants; Basic principles. Symposium “antioxidants and food”. Danone Institute, 2004,
[2] A. Favier, Oxidative stress. Conceptual and experimental interest in the understanding the mechanism of diseases and potential therapeutic. Meca Biochim, 2003, 25: 108-113.
[3] P. Léophonte, J.C. Guérin, F.X. Lebas, et al. Oxidative stress and BPCO. Roles in infections. Prevention. Med infect dis, 2006, 36: 245-252.
[4] J. Pincemail, J. Lecompte, E. Collart, et al. Ressoxidant, antioxidant et Physical exercice. Med Interne, 2003, 9: 56-59.
[5] A.B. Aliyu, M.A.Ibrahim, M. Aliyu, et al. Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis engl. (Araceae). Acta Pol Pharm Drug Res, 2013, 70:115-121.
[6] A. Augustyniak, G. Bartosz, A.C. Ipak, et al. Natural and synthetic antioxidants: An updated overview. Free Rad Res, 2010, 44: 1216–1262.
[7] R. Amarowicz, M. Naczk, F. Shahidi, Antioxidant activity of various fractions of non-tannin phenolics of canola hulls. J Agr Food Chem, 2000, 48: 2755-2759.
[8] J. Groff, S. Gropper, Advanced nutrition and human metabolism. 3th Edition Belmont: Wadsworth, 2000.
[9] G. Block, Vitamin C and cancer prevention: the epidemiologic evidence. Am J Clin Nutr Suppl, 1991, 53: 270-282.
[10] K.A. Youdim, J.A. Joseph. A possible emerging role of phytochemicals in improving age-related neurological dysfunctions: a multiplicity of effects. Free Rad Biol Med, 2001, 30: 583-594.
[11] I. Disengomoka, P. Delaveau, K. Sengeles, Medicinal plants used for child’s respiratory diseases in Zaire. Part II. J Ethnopharmcol, 1983, 8: 265-277.
[12] V.O. Oyetayo, Comparative Studies of the Phytochemical and Antimicrobial Properties of the Leaf, Stem and Tuber of Anchomanes difformis. J Pharm Toxicol 2007, 2: 407-410.
[13] H.M. Burkill, Useful plants of West Tropical Africa. vol. 11. Kew: Royal Botanical Gardens, 1994.
[14] K.K. Ajibesin, A.B. Ekpo, D.N. Bala, et al. Ethnobotanical survey of Akwa Ibom State of Nigeria. J Ethnopharmacol, 2007, 115: 387-408.
[15] J. Bero, V. Hannaert, Chataigne, et al. In vitro antitrypanosomal and antileishmanial activity of plants used in Benin in traditional medicine and bio-guided fractionation of the most active extract. J Ethnopharmacol, 2011, 137: 998-1002.
[16] G.E. Trease, W.C. Evans, Pharmacognosy. 13th edition. Bailliere Tindsll. Pp 176-186. 1989.
[17] S. Demiray, M.E. Pintado, P.M.L. Castro, Evaluation of Phenolic Profiles and Antioxidant Activi -ties of Turkish Medicinal Plants: Tilia argentea, Crataegi folium Leaves and Polygonum bistorta Roots’’. World Academy of Science, Engineering and Technology, 2009, 54: 312-317.
[18] M.S. Blois, Antioxidant determinattion by the use of stable free radical. Nature, 1958, 181: 1199-2000.
[19] H.O. Edeoga, D.E. Okwu, B.O. Mbaebie, Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotech, 2005, 4: 685-688.
[20] D. Huang, B. Ou, R.L. Prior, The chemistry behind antioxidant capacity assays. J Agr Food Chem, 2005, 5:1841-1856.
[21] L.B. Seef, K.L. Lindsay, B.R. Bacon, et al. Complementary and alternative medicine in chronic liver disease. Hepatology, 2001, 34: 595-603.


Table 1. Phytochemical analysis of *A. differmis* and *C. lutea* ethanolic extracts

| Phytochemical tests | *A. differmis leaves* | *A. differmis rhizomes* | *C. lutea fruits* |
|---|---|---|---|
| **Tannins** | - | + | + |
| **Saponins** | + | + | - |
| **Flavonoids** | + | + | - |
| **Phenols** | - | + | + |
| **Steroids** | + | - | - |
| **Alkaloids** | + | + | + |
| **Terpenoids** | - | + | - |
| **Coumarins** | - | + | + |
| **Anthraquinones** | + | + | - |
| **Anthracenic glycosides** | + | - | + |
| **Reducting substances** | + | + | + |

+: present; -: absent

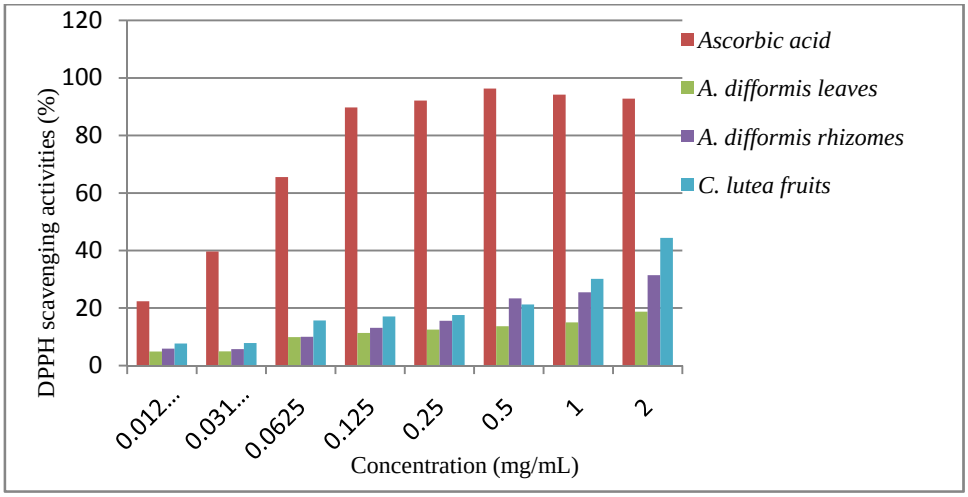


Figure 1: DPPH free radical activities (%) of *A. differmis* and *C. lutea* compared to that of ascorbic acid,