Asian Journal of Biochemistry, Genetics and Molecular Biology

Volume 16, Issue 10, Page 35-41, 2024; Article no.AJBGMB.107452
ISSN: 2582-3698

HPLC Profile of Bioactives and Antioxidant Potential in *Acalypha indica* Extract

Eboh Abraham Sisein [a*], Azibanasamesa D. C. Owaba [b] and Robert Owabhel Faith [a]

[a] Department of Biochemistry, Faculty of Basic Medical Science, Niger Delta University, Bayelsa State, Nigeria.
[b] Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajbgmb/2024/v16i10411

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107452

Original Research Article

Received: 01/09/2023
Accepted: 02/11/2023
Published: 14/10/2024

ABSTRACT

Acalypha indica is a plant found in many parts of Africa and Asia. The plant is used for the treatment of many respiratory diseases, management of cancers, diabetes and other diseases. The leaves of *Acalypha indica* were harvested, washed and shade dried. They were later extracted with methanol. The methanolic extract of *Acalypha indica* was subjected to DPPH radical scavenging assay, total antioxidant and HPLC quantification of phenols and flavonoids. The result showed a percentage yield of 5.72 %. Also the antioxidant capacity of *Acalypha indicia* based on the phosphomolybdenum method showed 13.60 ± 0.21 mg AAE/g extract. The DPPH radical

**Corresponding author: E-mail: ebohsisein@gmail.com; eboh.abraham@ndu.edu.ng;*

Cite as: *Sisein, Eboh Abraham, Azibanasamesa D. C. Owaba, and Robert Owabhel Faith. 2024. "HPLC Profile of Bioactives and Antioxidant Potential in Acalypha Indica Extract". Asian Journal of Biochemistry, Genetics and Molecular Biology 16 (10):35-41. https://doi.org/10.9734/ajbgmb/2024/v16i10411.*

scavenging potential of *Acalypha indica* showed that at 100 – 1000 µg/ml, the extract of *Acalypha indica* inhibited 7.2 ± 0.35 – 65.13 ± 1.53 % of DPPH radical as compared with standard gallic acid. The phenolics and flavonoids detected through HPLC are shown in Table 3. The phenolics and polyphenols with higher quantity in Extract of *Acalypha indica* are naringenin (169.91 mg/100 g), gallic acid (131.51 mg/100 g), rutin (76.72 mg/100 g), syringic acid (35.75 mg/100 g), ferulic acid (30.10 mg/100 g) and quercetin (11.36 mg/100 g). The results showed that *Acalypha indica* possesses antiradical, total antioxidant properties including a wide range of phenols and polyphenols. Therefore the extract can be a source of dietary supplement. This plant extract is a source of medicine if further research is applied.

Keywords: Acalypha indica; phytochemicals; DPPH; antioxidant; HPLC; flavonoids.

1. INTRODUCTION

Acalypha indica belongs to th Euphorbiaceae, it is common weed in Asia, Africa and South America [1]. The plant leaves and other parts are used against respiratory diseases like bronchitis, asthma and pneumonia [2]. The plant *Acalypha indica* is an erect shrub about 0.6 m tall with a few branches. *Acalypha indica* is an annual shrub found mostly in dump sites and fields in India, Africa and other continents [3,4]. It is seen across Asia, Africa and many parts of India [5]. *Acalypha indica* L. (family: *Euphorbiaceae*) is a shrub common in many parts of the globe. It is useful for the treatment of respiratory diseases, antibacterial and several other ailments [6,7].

There are scarce research works on phenolics profile using HPLC quantification, antiradical and antioxidant on the methanolic extract of *Acalypha indica*, that is why this research work was undertaken.

2. MATERIALS AND METHODS

2.1 Chemicals

DPPH radical, sodium phosphate, phosphomolybdate, sulfuric acid, ethanol, methanol, sodium hydroxide, quercetin, ascorbic acid and gallic acid all were purchased from Sigma-Aldrich (USA)

2.2 Plant Collection

Acalypha Indica fresh leaves were collected from Pharmacognosy Botanical Garden, Niger Delta University, Bayelsa State. The plant was identified and confirmed in the Department of Pharmacognosy, Niger Delta University, Bayelsa State.

2.3 Preparation of Methanolic Extract of *Acalypha Indica*

Acalypha indica leaves were harvested in large quantity. The leaves were washed to remove dirt. The leaves were shade dried for 14days (two weeks). The dried leaves were powdered using a blender which gave a dry weight of 74. 4 gram of which a part of it 50.7 grams was transferred into a jar and soaked with 700ml of methanol and kept for 72 hours (3 days) with occasional shaking. It was filtered using a filter paper to get the crude extract. The extract was concentrated. The paste formed was weighed and it was 2.9g. It was then stored at -4^0 C for further analysis.

2.4 Phytochemical Analysis

The Folin–Ciocalteu method as described by Singleton et al. [8] and Demiray et al. [9], was applied for phenol determination. Zhishen et al. [10] colorimetric method was adopted for total flavonoids. Alkaloid content the gravimetric method of Harborne (1973) was used

2.5 HPLC Quantification

Phenols and flavonoids were detected by HPLC from the extract of *Acalypha indica* [11].

2.6 Antioxidant Assays

Prieto et al. [12] colorimetric assay was used for total Antioxidant assay in *Acalypha indica* extract. 1, 1-diphenyl–2 picrylhydrazyl radical scavenging ability of the *Acalypha indica* extract was analyzed as described by Gyamfi et al. [13]. NO scavenging activity was determined by Marcocci and colleagues [14]. Iron Fe^{2+} chelating ability was quantified using Minotti and Aust [15] method and Puntel et al. [16].

2.7 Statistical Analysis

The results obtained were subjected to SPSS statistical analysis.

3. RESULTS AND DISCUSSION

Percentage Yield of Plant

Percentage yield (%) = 5.72

Acalypha indica is one of the plants with major medicinal properties for human health. The methanolic extract yielded 5.72 % .Antioxidants protects biomolecules from oxidising or neutralise free radicals [17]. Free radicals can be scavenged by antioxidants, reducing their impact. The total antioxidant value of *Acalypha Indica* was 13.60 ± 0.21 mgAAE/g [18]. The flavonoid content was found to be 18.89 ± 0.12 (mgQAE/g). Alkaloid content was found to be 12.3 ± 1.29 % and the phenolic content was found to be 25.01 ± 0.84 (mg GAE/g). This indicates a good amount of antioxidant and anti microbial properties compared to other plants like *Anethum graveolens L., Celsius Cristata* [19].

Table 1. Results of total antioxidant in extract of *Acalypha indica*

Sample	Total Antioxidant value
Acalypha Indica	13.60 ± 0.21 mgAAE/g

Values are mean ± S.D n = 3 determinants, AAE= Ascorbic Acid Equivalent

Extracts that reduce DPPH radical are good scavengers, the methanolic extract of *Acalypha Indica* increased from 7.2 ± 0.35 % at 100µg/ml to 65.13 ± 2.53% at 1000µg/ml. While the standard, Gallic acid also increased from 21.07±1.14 % at 100µg/ml to 70.19±1.04 % at 1000µg/ml. these increments in *Acalypha Indicai* and gallic acid were all concentration dependent, gallic acid pure in form becomes slightly more antiradical than *Acalypha indica* with respect to DPPH [20].

Using the NO radical scavenging assay, the methanolic extract of *Acalypha indicai* increased from 21.71 ± 2.15 at 100µg/ml to 96 ± 9.89 at 1000µg/ml. Its standard, Quacertin also increased from 3.80±0.87 at 100µg/ml to 76.16±16.78 at 1000µg/ml [21].

Ferulic acid was detected in large quantity 30.10 mg/100g extract of *Acalypha indica*. Ferulic acid is a phenol found in many plants [22] It is a powerful antioxidant that protect biological system from many reactive oxygen radicals [23]. Ferulic acid through its structure and chemistry protects PUFA [24]. The ferulic value in *Acalypha indica* is higher than that reported in *Peperomia pellucidia* 16.129 mg/100g of extract [20].

Quercetin is a flavonoid-flavonol-type largely a phytochemical [25]. Quercetin protected reproductive organs and cells in rat exposed to the toxicity of cadmium [26]. The amount of quercetin in *Acalypha indica* was11.36 mg/100g of extract this amount is lower than ferulic acid. Phenols and flavonoids are principal phytochemicals in plant and are responsible for many Medicinal claims of these plants. The amount of quercetin in *Peperomia pellucidia* was higher than that in *Acalypha indica* [21].

Gallic acid is a phenol usually found in plants [27]. It was reported to possess antihyperglycemic [28], antioxidant and anti-lipid peroxidation [29] properties. In the present study *Acalypha indica* possesses higher amount of gallic acid as shown in Table 3 (131.51 mg/100 g). This contributed to the many Medicinal properties of *Acalypha indica.*

Naringenin is a polyphenol, that protective the liver, anti-inflammatory and anti-mutagenic [30]. The amount of naringenin in *Acalypha indica* is the highest (169.91 mg/100 g) among the phenols and polyphenols as shown in Table 3, it is also higher than that in *Peperomia pellucidia* [20].

Syringic acid is a phenol that can be extracted from plants. Sini and Jun, [31] have showed the anti-inflammatory and pro-apoptosis potentials of syringic acid in Caco-2 cell lines. Syringic acid in *Acalypha indica* shows 35.75 mg/100 g which is higher than ferulic acid and quercetin Table 3.

The polyphenol Rutin is most abundant in *Ruta graveolens,* other plants and fruits. Rutin possesses anticancer, antioxidant and antidiabetic activities [32,33,34]. The amount of rutin in *Acalypha indica* was 76.72 mg/100 g of dry extract.

Table 2. Quantitative phytochemical content of *Acalypha indica*

Sample	Phytochemical	Phytochemical	Phytochemical content
Acalypha Indica	Total Phenol	Total Flavonoid	Total Alkaloid
	25.01 ± 0.84 (mgGAE/g)	18.89 ± 0.12 (mgQE/g)	12.3 ± 1.29%

Values are mean ± SDN=3 The values shows the phenolic, flavonoid and alkaloid content in methanolic extract of

Table 3. Percentage Fe^{2+} chelation and $NO^{\cdot}$ and DPPH by *Acalypha indica*

	% Iron chelation		% No scavenging		% DPPH Scavenging	
µg/ml	***Acalypha Indica***	**EDTA**	***Acalypha indica***	**Quercetin**	***Acalypha indica***	**Gallic acid**
100	19.67 ± 0.89	12.11± 1. 29	21.71 ± 1.15	3. 09 ± 0.78	7.2 ± 0.35	21. 07 ± 1. 14
200	36.17 ± 3.85	32.60 ± 1.76	31.05 ± 1.39	9.73 ± 1.18	9.83 ± 0.54	36. 07 ± 1. 79
400	48. 63 ± 1.69	58. 68 ± 2. 15	52.09 ± 0.44	34.16 ± 2.54	16.6 ± 2.69	42.69 ± 1.74
600	64. 45 ± 1. 21	70. 49 ±1.34	73.54 ± 1.61	50 .51 ± 1.59	26. 16 ± 2.21	53. 77 ± 2. 25
800	76.98 ± 1.45	79. 70 ± 0. 92	86.21 ± 0.04	66.90 ± 0.93	42.87 ± 2. 41	64. 58 ± 1. 04
	85.97 ± 2. 20	80.54 ± 1. 46	91.61 ± 0.89	76.65 ± 1.85	65.13 ± 1. 53	70. 19 ± 1. 04

Values are mean ± SD N=3 the chelation of Fe^{2+} and scavenging of $NO^{\cdot}$ and DPPH by Acalypha indica is concentration dependent

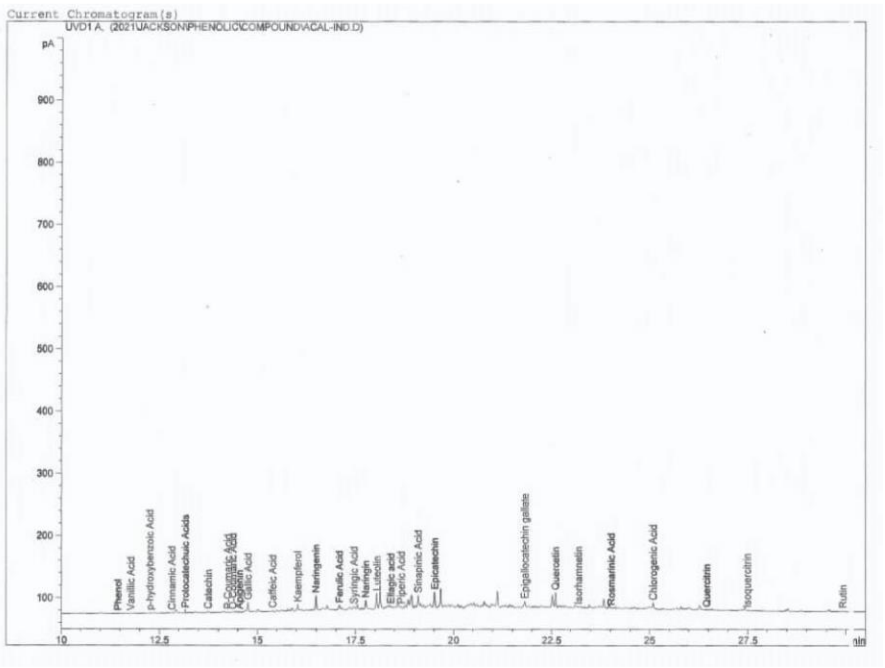

Fig. 1. chromatogram of phenolics and flavonoids in methanolic extract of *Acalypha indica*

Table 4. showing phenolics and flavonoids in methanolic extract of *Acalypha Indica*

Name of compound	Amount (mg/100g)	Retention time (min)
Phenol	6.97×10^{-4}	11.44
Vanillic acid	2.56×10^{-2}	11.77
P-hydroxybenzoic acid	1.54×10^{-3}	12.22
Cinnamic acid	5.73×10^{-4}	12.82
Protocatechuic acid	4.84×10^{-2}	13.16
Catechin	2.96×10^{-3}	13.74
P-coumaric acid	6.84×10^{-3}	14.24
O-coumaric acid	1.07×10^{-4}	14.37
Apigenin	7.04×10^{-4}	14.54
Gallic acid	131.51	14.76
Caffeic acid	59.72	15.39
Kaempferol	1.16×10^{-2}	16.03
Naringenin	169.91	16.49
Ferulic acid	30.10	17.09
Syringic acid	35.75	17.46
Naringin	2.56×10^{-1}	17.76
Luteolin	1.40×10^{-2}	18.05
Ellagic acid	4.57×10^{-3}	18.49
Piperic acid	1.76×10^{-5}	18.66
Sinapinic acid	1.95	19.10
Epicatechin	5.38×10^{-4}	19.51
Epigallocatechin gallate	2.36×10^{-3}	21.82
Quercetin	11.36	22.60
Isohamnetin	9.36×10^{-6}	23.18
Rosmarinic acid	2.92×10^{-3}	24.02
Chlorogenic acid	2.89×10^{-4}	25.08
Quercitrin	3.48×10^{-5}	26.45
Isoquercetrin	1.17×10^{-2}	27.48
Rutin	76.72	29.92

4. CONCLUSION

The plant *Acalypha Indica* is used ethnomedicinally for treatment of various diseases worldwide. The plant *Acalypha Indica* appears to be suitable for developing into different drugs that can be used to treat several diseases or disorders.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ramachandran J. Herbs of Siddha Medicine/The First 3D Book On Herbs. Murugan P Patthipagam, Chenna, India.2008:156.
2. Varier VPS. Indian medicinal plants: a compendium of 500 species Orient Longman.Publication, Madras, India.1996;134.
3. Muthukrishnan S, Prakathi P, Sivakumar T, Thiruvengadam M, Jayaprakash B, Baskar V, Rebezov M, Derkho M, Zengin G, Shariati MA. Bioactive components and health potential of endophytic micro-fungal diversity in medicinal plants. Antibiotics. 2022;11(11):1533.
4. Shanthi J, Seifunnisha O, Swathi R. Bioactive antimicrobial nanosystems: Enhancement of antimicrobial performance of Acalypha indica based ZnO nanomaterials and nonwettable activity. InAntimicrobial Nanosystems,Elsevier. 2023:103-115.
5. Burkill HM The useful plants of West Tropical Africa. Royal Botanic Gardens, Kew. United Kingdom. 1994;2:2-636.
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medical Plants. CSIR, New Delhi; 1956.
7. Govindarajan M, Jebanesan A, Reetha A, Amsath R, Pushpanathan T, Samidurai K, Antibacterial activity of *Acalypha indica L.* European Review for Medical and Pharmacological Sciences. 2008;12:299-302
8. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-179.
9. Demiray S, Pintado ME, Castro PM. Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tiliaargentea, Crataegi folium leaves and Polygonumbistortaroots. World Acad Sci Eng Technol. 2009;54:312–17.
10. Zhishen Y, Meugcheng T, Jianming W. Determination of flavonoids content in mulberry and their scavenging effect on superoxide radicals. Food chem.. 1999;64:555-9.
11. Luca SV, Miron A, Aprotosoaie AC, Mihai CT, Vochita G, Gherghel D, Ciocarlan N, Skalicka-Wozniak K. HPLC-DAD-ESI-Q-TOF-MS/MS profiling of Verbascum ovalifolium Donn ex Sims and evaluation of its antioxidant and cytogenotoxic activities. Phytochem. Anal. 2019;30:34–45.
12. Prieto P, Pineda M, Aguilar MM. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269:337-341.
13. Gyamfi MA, Yonamine, M, Aniya Y. Free radical scavenging activity of medicinal herb of Ghana: *Thonningiasan guinea* on experimentally induced liver injuries. Gen Pharmacol. 1999;32:661–667.
14. Marcocci I, Marguire JJ, Droy-lefaiz MT, Packer L. The nitric oxide scavenging properties of Ginkgo biloba extract. Biochem.Biophys. Res. Commun. 1994;201:748–755.
15. Minotti G, Aust SD. An investigation into the mechanism of citrate-Fe2+-dependent lipid peroxidation. Free RadicBiol Med. 1987;3:379–87.
16. Puntel RL, Nogueira CW, Rocha JBT. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro. Neurochemical Research. 2005;30:225-235
17. Eboh AS. Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences. 2014;2(2):110-118.
18. Rick-Leoniel N, Cedrick O, Joseph O, Louis I. Phytochemical screening antioxidant, anti-inflammatory Lophira procera. A. Chev (ochnacea) medicinal plant from Gabon 2017:80-86. DOI. 10.101/J.ebus.2017.11.003.
19. Nosheen A, Budsra M. Pythochemical Analysis and comprehensive evaluation if

antimicrobial and antioxidant properties of medicinal plant species. 2018;11(8):1223-1235.
DOI: 10.1061/ J.arabic. 2015.01.013.

20. Eboh AS, Robert FO, Owaba ADC. Phytochemicals, Radical Scavenging and Metal Chelating Properties of Methanolic Extract of *Senna Alata* IAR Journal of Agricultural Science and Food Research. 2022;2(3):1-3
21. Eboh AS, Robert FO, Wodu E. 2022 Phenolics and phytochemicals in methanolic extract of *Peperomia pellucidia* quantified by HPLC Open Access Research Journal of Life Sciences. 2022;03(02):059–062.
22. Mancuso C, Santangelo R. Ferulic acid: pharmacological and toxicological aspects, Food Chem. Toxicol. 2014. Available:https://doi.org/10.1016/j.fct.2013.12.024.
23. Kanski J, Aksenova M, Stoyanova A, Butterfield DA. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies, J. Nutr. Biochem. 2002 13 (5) 273–281. Available:https://doi.org/10.1016/S0955-2863(01)00215-7.
24. Rukkumani R, Aruna K, Suresh VP, Menon VP. Influence of ferulic acid on circulatory prooxidant - antioxidant status during alcohol and PUFA induced toxicity, J. Physiol. Pharmacol. 2004;55(3):551–561.
25. Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. Cancer Lett 2008;269:315–325.
26. Farombi EO, Adedara IA, Akinrinde SA, Ojo OO, Eboh AS. Protective effects of kolaviron and quercetin on cadmium-induced testicular damage and endocrine pathology in rats Andrologia. 2012;1–12.
27. Singh MP, Gupta A, Sisodia SS. Gallic Acid: Pharmacogical promising lead molecule: A Review International Journal of Pharmacognosy and Phytochemical Research. 2018:10(4):132-138.
28. Prince MSP., Kumar MR, Selvakumari JC. Effect of gallic acid on brain lipid peroxide, lipid metabolism in streptozotocin-induced diabetic wistar rats; Journal of Biochemical and molecular Toxicology. 2011;25(2):101-107.
29. Khanh VD, Chang MK, Ann WK. Gallic acid regulates body body weight and glucose homeostasis through AMPK activation. Endocrinology. 2015;156(1):157-168
30. Venkateswara RP, Kiran SDVS, Rohini P, Bhagyasree P. Flavonoid: A review on Naringenin. Journal of Pharmacognosy and Phytochemistry. 2017;6(5):2778-2783.
31. Sini X, Jun X. Protective effects of syringic acid on inflammation, apoptosis and intestinal barrier function in Caco-2 cells following oxygen-glucose deprivation/reoxygenation-induced injury, Experimental and Therapeutic Medicine. 2022;23:66 1-7.
32. Lin JP, Yang JS, Lin JJ, Lai KC,. Lu HF, Ma CY, Wu RSC, Wu KC, Chueh FS, Wood WG, Chung JG. Rutin inhibits human leukemia tumor growth in a murine xenograft model. Environmental in vivo Toxicology. 2012;27(8):480-484.
33. Yang J, Guo J, Yuan J. In vitro antioxidant properties of rutin. LWT – Food Science and Technology. 2008;41(6):1060-1066.
34. Sattanathan K, Dhanapal CK, Umarani R, Manavalan R. Beneficial health effects of rutin supplementation in patients with diabetes mellitus. Journal of Applied Pharmaceutical Science, 2011;01(08):227-231.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107452