HPLC PROFILE OF BIOACTIVES AND ANTIOXIDANT POTENTIAL IN*Acalyphaindica* EXTRACT

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 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.

Keyword: Acalypha indica, phytochemicals, DPPH, antioxidant, HPLC, flavonoids

INTRODUCTION

Acalyphaindica belongs to thEuphorbiaceae, it is common weed in Asia, Africa and South America (Ramachandran, 2008). The plant leaves and other parts are used against respiratory diseases like bronchitis, asthma and pneumonia (Varier, 1996). 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 (Muthukrishnan et al., 2022; Shanthi et al., 2023). It is seen across Asia, Africa and many parts of India (Burkill, 1994). *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 (Chopra *et al.*, 1956, Govindarajan *et al.*, 2008).

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.

MATERIALS AND METHODS

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)

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.

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.

Phytochemical analysis

The Folin–Ciocalteu method as described by Singleton *et al.*,1999 and Demiray *et al.*,2009,was applied for phenol determination. Zhishen*et al.*, 1999colorimetric method was adopted for total flavonoids. Alkaloid content the gravimetric method of Harborne (1973) was used

HPLC QUANTIFICATION

Phenols and flavonoids were detected by HPLC from the extract of *Acalypha indica* (Luca *et al.*, 2019).

ANTIOXIDANT ASSAYS

Prieto *et al.*,1999 colorimetric assay was used for total Antioxidant assay in*Acalypha indica* extract. 1, 1-diphenyl–2 picrylhydrazyl radical scavengingability of the *Acalypha indica* extract was analyzed as described by Gyamfi *et al.*, 1999. NO scavenging activitywas determined byMarcocci and colleagues 1994. Iron Fe^{2+}chelating ability was quantified using Minotti and Aust 1987 method and Puntel *et al.*, 2005.

Statistical analysis

The results obtained were subjected to SPSS statistical analysis.

RESULTS

Percentage Yield of Plant

Percentage yield (%) = 5.72%

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

Table 2 Quantitative Phytochemical content of *Acalypha indica*

Sample	**Phytochemical**	**Phytochemical**	**Phytochemical**
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 ± SD N=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*

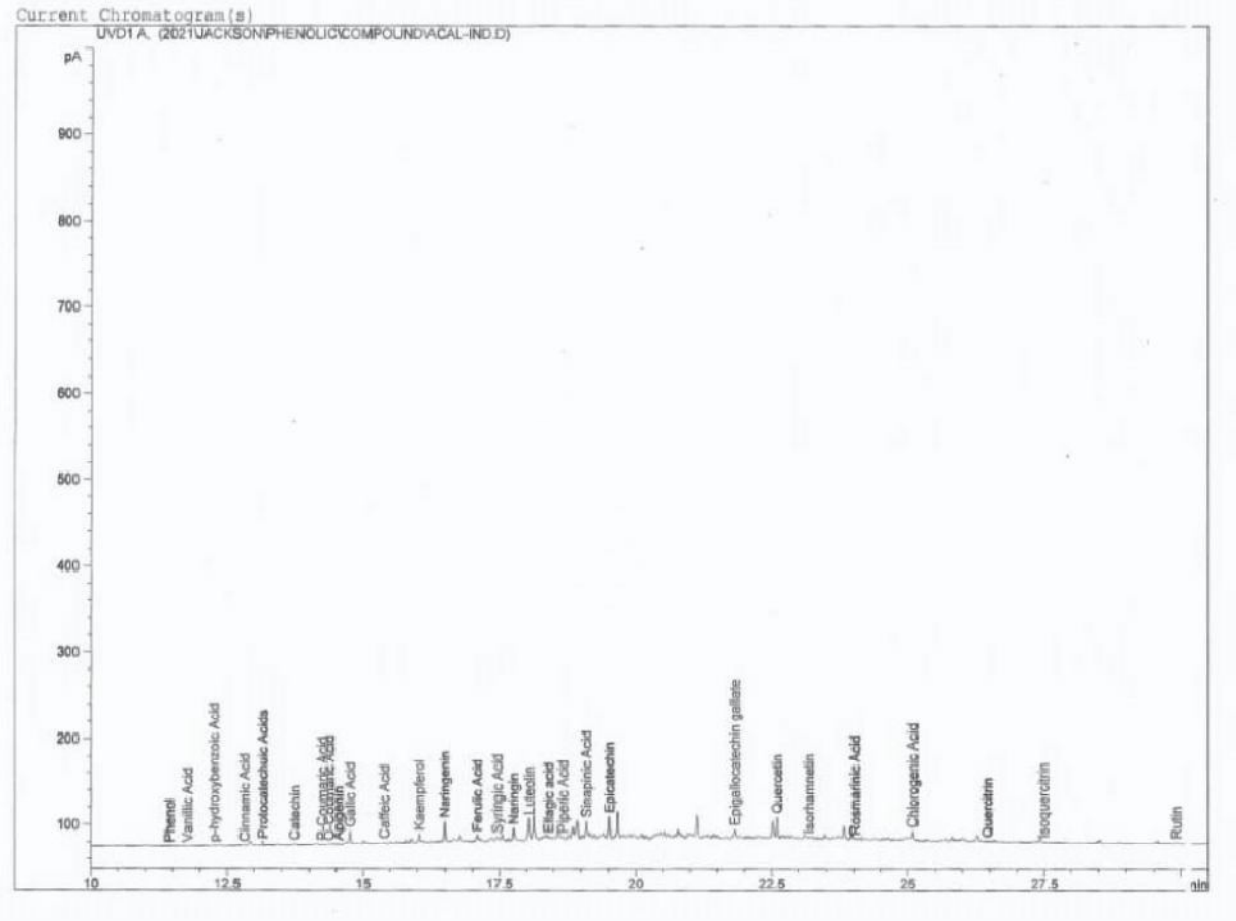

Table 4 showing phenolics and flavonoids in methanolic extract of *Acalypha Indica*

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

DISCUSSION

Acalypha indica is one of the plants with major medicinal properties for human health. The methanolic extract yielded 5.72 % .Antioxidants protectsbiomolecules from oxidising or neutralise free radicals (Eboh, 2014). Free radicals can be scavenged by antioxidants, reducing their impact. The total antioxidant value of *Acalypha Indica* was 13.60 ± 0.21 mgAAE/g Rick *et al.,* 2017.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 (* Nosheen et al., 2018).

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 *AcalyphaIndicai* and gallic acid were all concentration dependent, gallic acid pure in form becomes slightly more antiradical than *Acalypha indica* with respect to DPPH (Eboh *et al.,* 2022).

Using the NO radical scavenging assay, the methanolic extract of *Acalyphaindicai*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(Eboh *et al.,* 2022).

Ferulic acid was detected in large quantity 30.10 mg/100g extract of *Acalypha indica*. Ferulic acid is a phenol found in many plants (Mancuso and Santangelo, 2014) It is a powerful antioxidant that protect biological system from many reactive oxygen radicals(Kanski *et al.,* 2002). Ferulic acid through its structure and chemistry protects PUFA (Rukkumani *et al.,* 2004). The ferulic value in *Acalypha indica* is higher than that reported in *Peperomiapellucidia*16.129 mg/100g of extract (Eboh *et al.,* 2022).

Quercetin is a flavonoid-flavonol-type largely a phytochemical (Murakami *et al.,* 2008). Quercetin protected reproductive organs and cells in rat exposed to the toxicity of cadmium (Farombi*et al.,*2012). 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 *Peperomiapellucidia* was higher than that in *Acalypha indica*(Eboh *et al.,* 2022).

Gallic acid is a phenol usually found in plants (Singh *et al.,* 2018). It was reported to possess antihyperglycemic (Prince *et al.,* 2011), antioxidant and anti-lipid peroxidation (Khanh *et al.,* 2015) 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 (Venkateswara*et al*., 2017). 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 *Peperomiapellucidia*(Eboh*et al.,* 2022).

Syringic acid is a phenol that can be extracted from plants. Sini and Jun, 2021 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 (Lin *et al.,* 2012, Yang *et al.,* 2008 and Sattanathan *et al.,* 2011). The amount of rutin in *Acalyphaindica* was 76.72 mg/100 g of dry extract.

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.

REFERENCES

Sini, X., Jun, X (2022). 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, 23: 66 1-7.

Yang, J., Guo, J., Yuan, J (2008). In vitro antioxidant properties of rutin. LWT - Food Science and Technology, 41(6): 1060-1066.

Sattanathan, K., Dhanapal, C.K., Umarani, R., Manavalan,R (2011). Beneficial health effects of rutin supplementation in patients with diabetes mellitus. Journal of Applied Pharmaceutical Science, 01 (08): 227-231.

Lin, J.P., Yang, J.S., Lin, J.J., Lai, K.C,. Lu, H.F., Ma, C.Y., Wu, R.S.C.,Wu, K.C., Chueh, F.S, Wood, W.G., Chung, J.G (2012). Rutin inhibits human leukemia tumor growth in a murine xenograft model . Environmental in vivo Toxicology, 27(8): 480-484.

Venkateswara, R.P., Kiran, S.D.V.S., Rohini, P., Bhagyasree, P (2017).Flavonoid: A review on Naringenin. Journal of Pharmacognosy and Phytochemistry; 6(5): 2778-2783

Prince, M.S.P., Kumar, M.R., Selvakumari, J.C. (2011). Effect of gallic acid on brain lipid peroxide, lipid metabolism in streptozotocin-induced diabetic wistar rats; Journal of Biochemical and molecular Toxicology 25(2):101-107.

Singh, M.P., Gupta, A., Sisodia, S.S. (2018). Gallic Acid: Pharmacogical Promising Lead Molecule: A Review International Journal of Pharmacognosy and Phytochemical Research; 10(4); 132-138.

Khanh, V.D., Chang, M.K., Ann, W.K. (2015). Gallic acid regulates body body weight and glucose homeostasis through AMPK activation. Endocrinology; 156(1):157-168

Prieto, P., Pineda, M., Aguilar, M.M.(1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 269:337-341.

Rick-Leoniel, N., Cedrick, O., Joseph, O., and Louis, I. (2017). Phytochemical screening antioxidant, anti-inflammatory Lophiraprocera. A. Chev (ochnacea) medicinal plant from Gabon. pp 80-86. Doi. 10.101/J.ebus.2017.11.003.

Gyamfi, M.A., Yonamine, M.,Aniya,Y.(1999). Free radical scavenging activity of medicinal herb of Ghana: *Thonningiasanguinea* on experimentally induced liver injuries. *Gen Pharmacol*. 32:661–667.

Mancuso, C., Santangelo, R. (2014). Ferulic acid: pharmacological and toxicological aspects, Food Chem. Toxicol. (2014), https://doi.org/10.1016/j.fct.2013.12.024.

Kanski,J., Aksenova,M., Stoyanova, A., Butterfield, D.A (2002). 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. 13 (5) 273–281, https://doi.org/10.1016/S0955-2863(01)00215-7.

Puntel,R..L, Nogueira,C.W., and Rocha, J.B.T (2005).Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain in vitro.*Neurochemical Research*, 30 pp. 225-235

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299: 152-179.

Eboh, A.S., Robert, F.O., And Owaba, A.D.C (2022). Phytochemicals, Radical Scavenging and Metal Chelating Properties of Methanolic Extract of *Senna Alata* IAR Journal of Agricultural Science and Food Research vol 2 (3) 1-3

Eboh, A.S (2014) Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences; 2(2): 110-118.

Rukkumani, R., Aruna, K., Suresh, V.P., Menon, V.P (2004). Influence of ferulic acid on circulatory prooxidant - antioxidant status during alcohol and PUFA induced toxicity, J. Physiol. Pharmacol. 55 (3) 551–561.

Eboh, A.S., Robert, F.O., Wodu, E (2022). Phenolics and phytochemicals in methanolic extract of *Peperomiapellucidia*quantified by HPLC Open Access Research Journal of Life Sciences, 2022, 03(02), 059–062.

Murakami, A., Ashida, H., Terao, J. (2008) Multitargeted cancer prevention by quercetin. Cancer Lett 269:315–325.

Farombi, E.O.,Adedara, I.A.,Akinrinde, S.A.,Ojo, O.O., Eboh, A.S.(2012). Protective effects of kolaviron and quercetin on cadmium-induced testicular damage and endocrine pathology in rats Andrologia, xx, 1–12.

Chopra, R.N., Nayar, S.L., Chopra,I.C. (1956). Glossary of Indian Medical Plants. CSIR, New Delhi.

Govindarajan, M., Jebanesan, A., Reetha, A., Amsath, R., Pushpanathan, T., Samidurai, K.,(2008) Antibacterial activity of *Acalypha indica L.* European Review for Medical and Pharmacological Sciences; 12: 299-302

Burkill,H.M. (1994). The useful plants of West Tropical Africa. Royal Botanic Gardens, Kew. United Kingdom 2: 2-636.

Luca, S.V., Miron, A., Aprotosoaie, A.C., Mihai, C.T., Vochita, G., Gherghel, D., Ciocarlan, N., Skalicka-Wozniak, K.(2019). HPLC-DAD-ESI-Q-TOF-MS/MS profiling of Verbascum ovalifoliumDonn ex Sims and evaluation of its antioxidant and cytogenotoxicactiv ities. Phytochem. Anal. 30, 34–45.

Varier, V.P.S. (1996). Indian medicinal plants: a compendium of 500species Orient Longman.Publication, Madras, India, p. 134.

Ramachandran, J. (2008). Herbs of Siddha Medicine/The First 3D Book On Herbs. Murugan PPatthipagam, Chenna, India, p. 156.

Demiray, S., Pintado, M.E., Castro, P.M.(2009) Evaluation of phenolic profiles and antioxidant activities of Turkish medicinal plants: Tiliaargentea, Crataegi folium leaves and Polygonumbistortaroots.WorldAcadSciEngTechnol 54:312–17.

Marcocci, I., Marguire, J.J., Droy-lefaiz, M.T., Packer, L.(1994) The nitric oxide scavenging properties of Ginkgo biloba extract. Biochem.Biophys. Res. Commun. 201, 748–755.

Minotti, G., Aust, S.D. (1987) An investigation into the mechanism of citrate-Fe2+-dependent lipid peroxidation. Free RadicBiol Med;3:379–87.

Zhishen, Y., Meugcheng, T., Jianming, W.(1999). Determination of flavonoids content in mulberry and their scavenging effect on superoxide radicals. Food chem, 64: 555-9.

Nosheen, A., Budsra, M. (2018) Pythochemical Analysis and comprehensive evaluation if antimicrobial and antioxidant properties of medicinal plant species .11 (8)pp 1223-1235. Doi: 10.1061/ J.arabic. 2015.01.013.

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 Nov 2;11(11):1533.

Shanthi J, Seifunnisha O, Swathi R. Bioactive antimicrobial nanosystems: Enhancement of antimicrobial performance of Acalyphaindica based ZnOnanomaterials and nonwettable activity. InAntimicrobialNanosystems 2023 Jan 1 (pp. 103-115). Elsevier.