

# HPLC PROFILE OF BIOACTIVES AND ANTIOXIDANT POTENTIAL IN *Acalypha indica* 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 indica* based on the phosphomolybdenum method showed  $13.60 \pm 0.21$  mg AAE/g extract. The DPPH radical scavenging potential of *Acalypha indica* showed that at 100 – 1000  $\mu\text{g/ml}$ , the extract of *Acalypha indica* inhibited  $7.2 \pm 0.35 - 65.13 \pm 1.53$  % 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

*Acalypha indica* belongs to the Euphorbiaceae, 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. 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 14 days (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  $-4^{\circ}$  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.*, 1999 colorimetric 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 scavenging ability of the *Acalypha indica* extract was analyzed as described by Gyamfi *et al.*, 1999. NO scavenging activity was determined by Marcocci 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
<i>Acalypha Indica</i>	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
<i>Acalypha Indica</i>	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*

$\mu$ g/ml	% IRON CHELATION		% $NO^{\cdot}$ scavenging		% DPPH Scavenging	
	<i>Acalypha Indica</i>	EDTA	<i>Acalypha indica</i>	Quercetin	<i>Acalypha indica</i>	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<sup>2+</sup> and scavenging of NO<sup>•</sup> and DPPH by *Acalypha indica* is concentration dependent

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

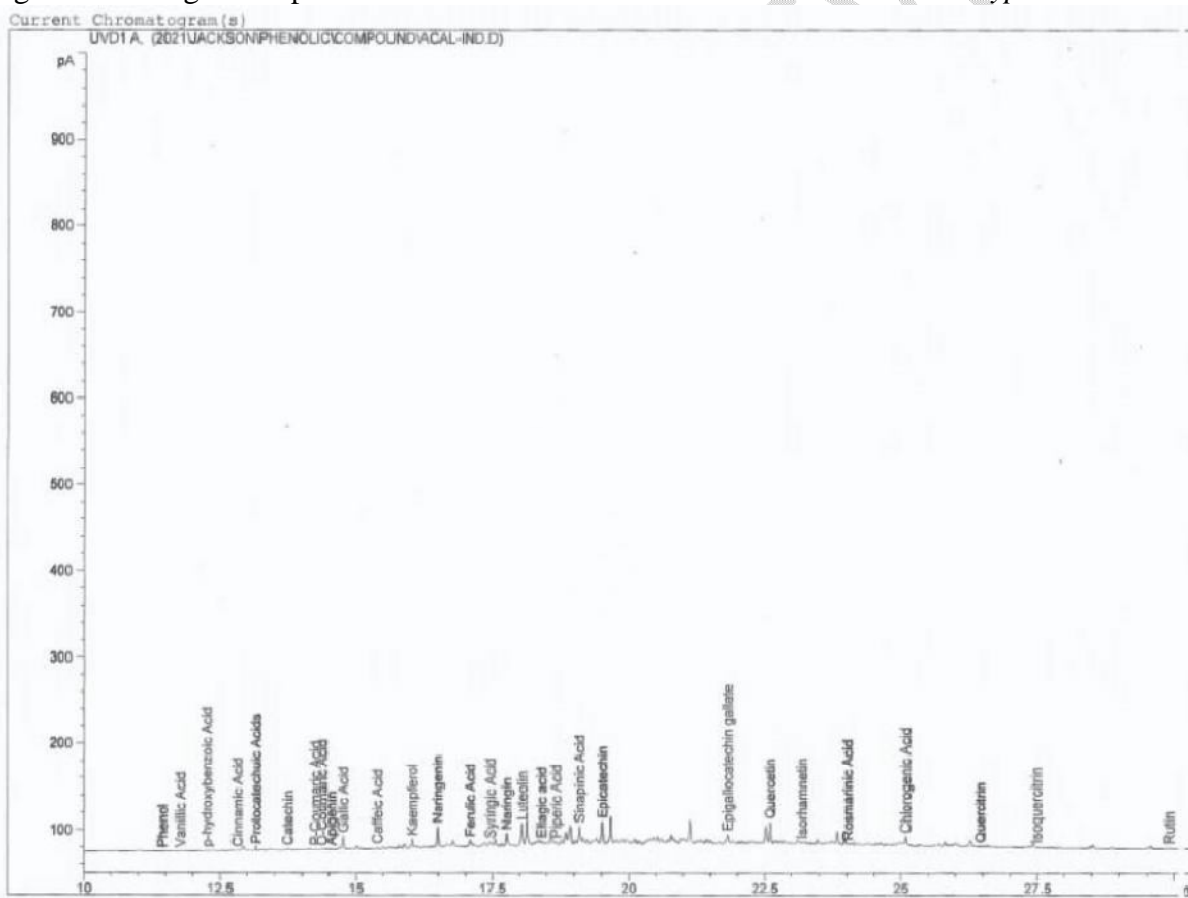


Table 4 showing phenolics and flavonoids in methanolic extract of *Acalypha Indica*

Name of compound	Amount (mg/100g)	Retention time (min)
Phenol	6.97 x 10 <sup>-4</sup>	11.44
Vanillic acid	2.56 x 10 <sup>-2</sup>	11.77

P-hydroxybenzoic acid	$1.54 \times 10^{-3}$	12.22
Cinnamic acid	$5.73 \times 10^{-4}$	12.82
Protocatechuic acid	$4.84 \times 10^{-2}$	13.16
Catechin	$2.96 \times 10^{-3}$	13.74
P-coumaric acid	$6.84 \times 10^{-3}$	14.24
O-coumaric acid	$1.07 \times 10^{-4}$	14.37
Apigenin	$7.04 \times 10^{-4}$	14.54
Gallic acid	131.51	14.76
Caffeic acid	59.72	15.39
Kaempferol	$1.16 \times 10^{-2}$	16.03
Naringenin	169.91	16.49
Ferulic acid	30.10	17.09
Syringic acid	35.75	17.46
Naringin	$2.56 \times 10^{-1}$	17.76
Luteolin	$1.40 \times 10^{-2}$	18.05
Ellagic acid	$4.57 \times 10^{-3}$	18.49
Piperic acid	$1.76 \times 10^{-5}$	18.66
Sinapinic acid	1.95	19.10
Epicatechin	$5.38 \times 10^{-4}$	19.51
Epigallocatechin gallate	$2.36 \times 10^{-3}$	21.82
Quercetin	11.36	22.60
Isohamnetin	$9.36 \times 10^{-6}$	23.18
Rosmarinic acid	$2.92 \times 10^{-3}$	24.02
Chlorogenic acid	$2.89 \times 10^{-4}$	25.08
Quercitrin	$3.48 \times 10^{-5}$	26.45
Isoquercetrin	$1.17 \times 10^{-2}$	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 protects biomolecules 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  $13.60 \pm 0.21$  mgAAE/g Rick *et al.*, 2017. The flavonoid content was found to be  $18.89 \pm 0.12$  (mgQAE/g). Alkaloid content was found to be  $12.3 \pm 1.29\%$  and the phenolic content was found to be  $25.01 \pm 0.84$  (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  $7.2 \pm 0.35$  % at  $100\mu\text{g/ml}$  to  $65.13 \pm 2.53\%$  at  $1000\mu\text{g/ml}$ . While the standard, Gallic acid also increased from  $21.07 \pm 1.14$  % at  $100\mu\text{g/ml}$  to  $70.19 \pm 1.04$  % at  $1000\mu\text{g/ml}$ . these increments in *Acalypha Indica* 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 *Acalypha indica* increased from  $21.71 \pm 2.15$  at  $100\mu\text{g/ml}$  to  $96 \pm 9.89$  at  $1000\mu\text{g/ml}$ . Its standard, Quercetin also increased from  $3.80 \pm 0.87$  at  $100\mu\text{g/ml}$  to  $76.16 \pm 16.78$  at  $1000\mu\text{g/ml}$  (Eboh *et al.*, 2022).

Ferulic acid was detected in large quantity  $30.10\text{ mg}/100\text{g}$  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 *Peperomia pellucida*  $16.129\text{ mg}/100\text{g}$  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 (Farombiet *et al.*, 2012). The amount of quercetin in *Acalypha indica* was  $11.36\text{ mg}/100\text{g}$  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 pellucida* 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 ( $131.51\text{ mg}/100\text{ g}$ ). 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 ( $169.91\text{ mg}/100\text{ g}$ ) among the phenols and polyphenols as shown in table 3, it is also higher than that in *Peperomia pellucida* (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  $35.75\text{ mg}/100\text{ g}$  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 *Acalypha indica* was  $76.72\text{ mg}/100\text{ g}$  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.

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