

Phytochemical composition and antioxidant property of *Acalypha indica* L. fractions

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Abstract

Oxidative stress has been demonstrated to significantly contribute to the onset of several disease processes in the human body. Natural antioxidants are efficient in safeguarding the human body against oxidative damage caused by free radicals. Plants are a significant source of numerous pharmaceuticals from various categories that are recognized for alleviating diverse ailments, including oxidative stress. *Acalypha indica* belongs to the Euphorbiaceae family. Extracts from the leaves of this plant have been documented to exhibit diverse biological activity. The current work involved the fractionation of methanolic leaf extract of *Acalypha indica* L. into ethyl acetate and n-butanol. The extracted extract and fractions underwent preliminary phytochemical screening and investigation of antioxidant activity. In vitro tests were used to check the antioxidant activities. These included DPPH, O₂⁻, and OH⁻ scavenging activities, as well as nitric oxide free radical scavenging activity, reducing power assay, and metal chelating activity. The antioxidant activities were compared to those of butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, and ethylenediaminetetraacetic acid (EDTA). In vitro tests showed that the methanolic, ethyl acetate, and n-butanolic parts of *Acalypha indica* had strong antioxidant effects. Significant free radical scavenging

activity, robust reducing power, and effective ion chelating capabilities were noted. Based on the results of this study, the methanolic leaf extract of *Acalypha indica* might be a good way to get natural antioxidants.

Key words : *Acalypha indica*, antioxidant properties, Oxidative stress, phytochemical screening, characterization.

Reactive oxygen species (ROS), including free radicals such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), have garnered increased interest. Radicals (O_2^-), hydroxyl radicals (OH^-), and non-radicals. Pathological situations result in the overproduction of reactive oxygen species (ROS), culminating in oxidative stress. Numerous disorders are attributable to oxidative stress, including metabolic inflammatory¹ and cardiovascular disease, tumor proliferation, dermal aging³, cancer², and Alzheimer's disease⁴. Antioxidants are crucial in safeguarding the human body from harm caused by reactive oxygen species. Antioxidants mitigate the harmful effects of reactive oxygen species (ROS). Antioxidants can disrupt the oxidation process by engaging with free radicals⁵. Still, research shows that man-made antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) can cause cancer. There has been a growing interest in identifying natural antioxidants that can safeguard the human body from free radicals and impede the advancement of certain chronic diseases. Researchers are diligently seeking innovative natural antioxidants derived from plants. There are many phytochemicals found naturally in plants that protect against oxidative damage caused by reactive oxygen species.

Acalypha indica L. is a tiny annual erect herbaceous plant that attains heights of up to

0.6 m, 0.35–0.75 m and 0.3–1.0 m, prevalent in equatorial regions across the continents of Asia, Africa, Europe, North America, South America, and Australia^{6,7}. This plant is referred to as Muktavarchaa, Haritamanjari in Ayurveda, Kuppu, Khokali in Hindi, Arittamanjarie in Sanskrit, Khokalee in Marathi⁷, and Muktajhuri in Bengali⁸.

Collection of the plant material and preparation of extracts :

We obtained *Acalypha indica* leaves from local fields and shaded dried them at ambient temperature for 2–3 days. The dried leaves were subsequently ground in a mixer to get a coarse powder for extraction. The *A. indica* leaves were extracted with a Soxhlet extractor. One hundred grams of the powdered substance were extracted with 500 mL of methanol for 24 hours. After extraction, the solvent was removed via a rotary flash evaporator. The extract residue was dried in a desiccator, and the resulting extract was stored in an airtight container at 4°C for future use. The extract underwent a first phytochemical analysis and in vitro antioxidant assessments.

Phytochemical screening of methanolic leaf extract:

Following standard procedures, the methanolic leaf extract of *A. indica* was tested

for phytochemicals to find phytoconstituents like proteins, reducing sugars, oils and fats, cardiac glycosides, tannins, saponins, phenols, flavonoids, phytosterols, alkaloids, steroids, catechin, anthraquinone, and terpenoids⁹.

Screening for antioxidant activities :

There were several tests done on methanolic leaf extract from *A. indica* to see how well it worked as an antioxidant. These included DPPH, superoxide anion radical (O_2^-) and hydroxyl radical (OH^-) scavenging activities, nitric oxide free radical scavenging activity, reducing power assay, and metal

chelation. The results were compared to positive controls like ascorbic acid, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA). The phytochemical analysis of the methanolic leaf extract of *A. indica* shows the presence of reducing sugars, proteins, phenols, alkaloids, flavonoids, saponins, tannins, and cardiac glycosides (Table-1). Total flavonoids content estimation of MEA and its fractions NBA and EAA was found to be 28.42, 12.58 and 31.75, respectively (Table-2). Total flavonoids content estimation of MEA and its fractions NBA and EAA was found to be 1.47, 1.09 and 1.76. respectively (Table-3).

Table-1. Preliminary phytochemical profile of ME and its fraction

TESTS	RESULTS		
	MEA	NBA	EAA
1. Carbohydrates			
• Molisch's test	Positive	Positive	Positive
• Fehling's test	Positive	Positive	Positive
2. Proteins			
• Millon's test	Negative	Negative	Negative
• Biuret test	Negative	Negative	Negative
3. Essential oil	Negative	Negative	Negative
4. Steroids			
• Salkowski test	Positive	Positive	Negative
• Libermann-burchard test	Positive	Positive	Negative
5. Phenolic compounds			
• Potassium permanganate	Positive	Positive	Positive
• Ferric chloride	Positive	Positive	Positive
6. Flavonoides			
• Lead acetate test	Positive	Positive	Positive
• Shinoda test	Positive	Positive	Positive
7. Alkaloids			
• Mayer's test	Negative	Negative	Negative
• Wagner's test	Negative	Negative	Negative

Table-2. Total phenolic content estimation of ME and its fractions

Sample	Conc. ($\mu\text{g/ml}$)	Absorbance [#]	(μg gallic acid/ g of sample)
Gallic acid Std curve	10	0.12 ± 0.018	-
	20	0.29 ± 0.011	-
	40	0.58 ± 0.017	-
	60	0.82 ± 0.013	-
	80	1.03 ± 0.021	-
	100	1.30 ± 0.014	-
MEA	100	0.37 ± 0.023	28.42
NBA	100	0.18 ± 0.013	12.58
EAA	100	0.41 ± 0.019	31.75

(# the values are expressed as mean \pm SD; n=3)

Table-3. Total flavonoids content estimation of MEA and Its fractions

Sample	Conc. ($\mu\text{g/ml}$)	Absorbance [#]	(μg quercetin/g of sample)
Quercetin Std curve	10	0.08 ± 0.012	-
	20	0.11 ± 0.017	-
	40	0.17 ± 0.013	-
	60	0.24 ± 0.009	-
	80	0.29 ± 0.018	-
	100	0.34 ± 0.015	-
MEA	100	0.09 ± 0.008	1.47
NBA	100	0.073 ± 0.011	1.09
EAA	100	0.109 ± 0.013	1.76

(# the values are expressed as mean \pm SD; n=3)

We found that the methanolic leaf extract of *A. indica* is excellent at getting rid of free radicals. This benefit was seen in the DPPH test, the superoxide anion radical (O_2^-) test, the hydroxyl radical (OH^-) test, and the nitric oxide free radical test. The extract exhibited considerable reducing power and metal chelating activity. Figures 2–7 show that the methanolic extract had an effect that was like that of common antioxidants like ascorbic

acid, EDTA, BHT, and BHA. The research indicated that the methanolic leaf extract of *A. indica* has a lot of antioxidant power¹⁰.

Free radicals significantly contribute to the pathogenesis of various human diseases, including cancer, rheumatoid arthritis, and cardiovascular disorders. Plants containing diverse phytochemicals are a primary source of natural antioxidants that may mitigate the

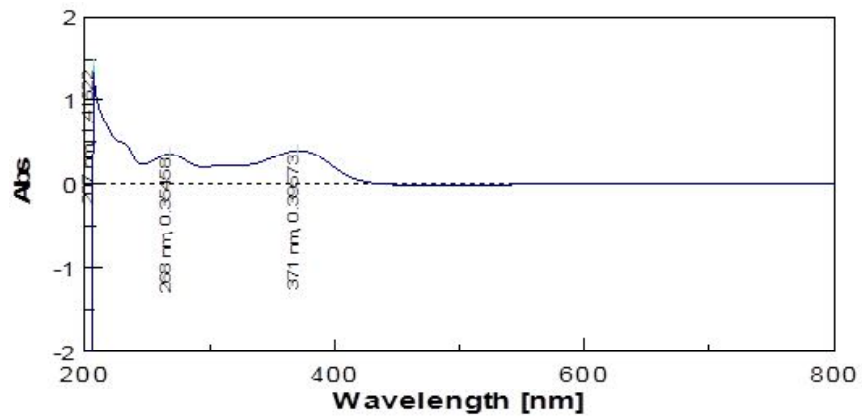
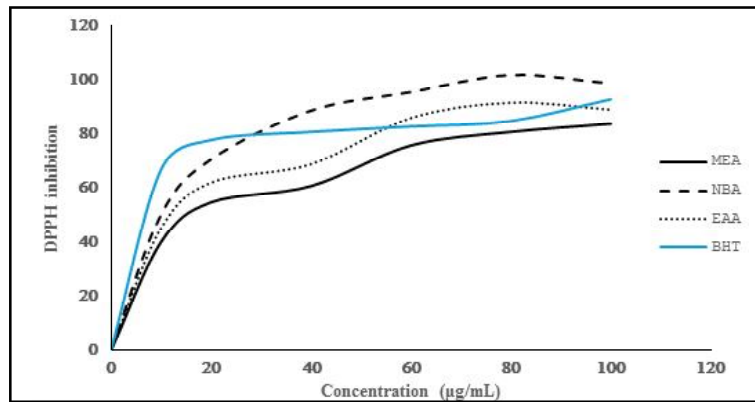
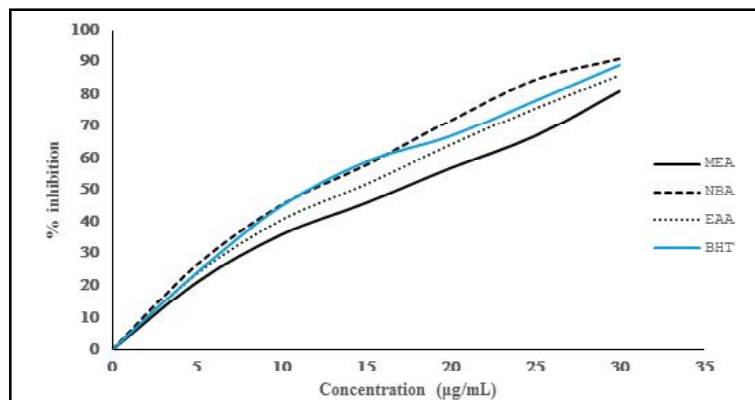


Figure 1. UV spectrum of MEA

Fig. 2. DPPH radical scavenging activity of butylated hydroxytoluene (BHT) and methanolic leaf extract (ME) of *A. indica*Fig. 3. Superoxide anion (O_2^-) radical scavenging activity of butylated hydroxy-toluene (BHT) and methanolic leaf extract *A. indica*

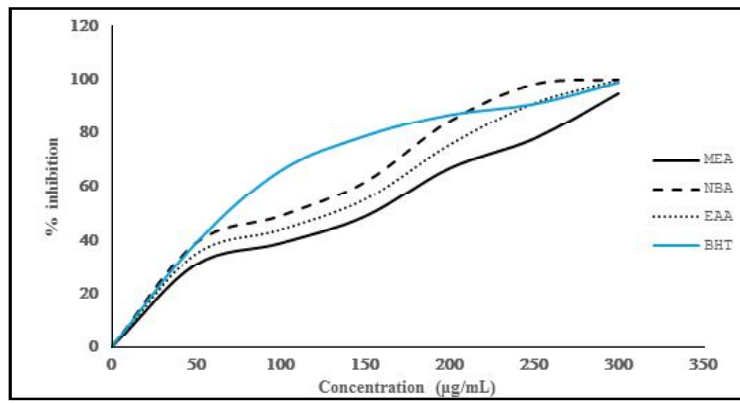


Fig. 4. Hydroxyl (OH) radical scavenging activity of butylated hydroxytoluene (BHT) and methanolic leaf extract (ME) of *A. indica*

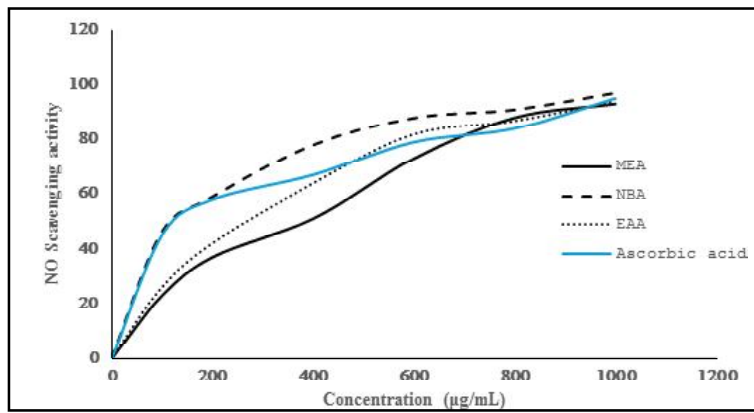


Fig. 5. Nitric oxide (NO) radical scavenging activity of ascorbic acid and methanolic leaf extract (ME) of *A. indica*

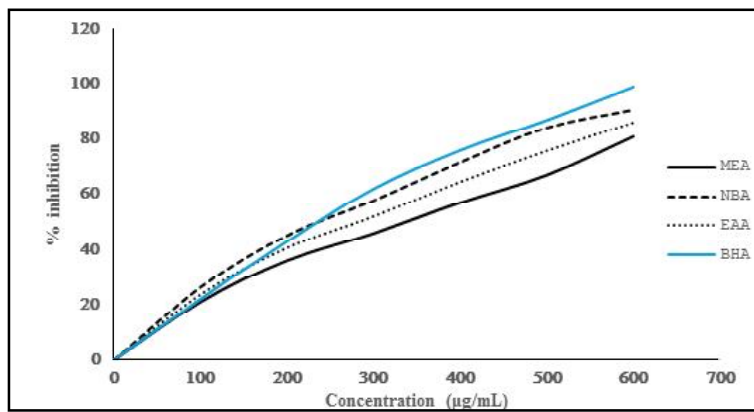


Fig. 6. Reducing power assays of butylated hydroxyanisole (BHA) and methanolic leaf extract (ME) of *A. indica*

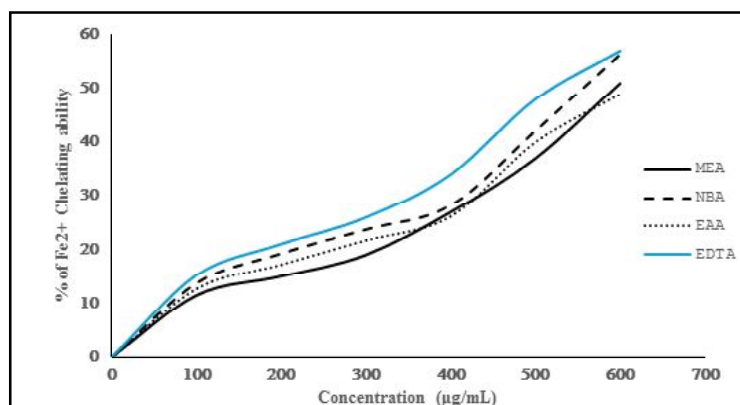


Fig. 07. Fe^{2+} metal chelating ability of Ethylenediaminetetraacetic acid (EDTA) and methanolic leaf extract (ME) of *A. indica*

pathophysiology of free radicals¹⁰. The findings of this study clearly showed that the methanolic leaf extract of *A. indica* has strong antioxidant properties. The phytochemical analysis of the methanolic leaf extract of *A. indica* showed that it contains phenolic components, mostly flavonoids and tannins, which are very good at fighting free radicals. These chemicals may account for the favourable antioxidant properties discovered in the methanolic leaf extract of *A. indica*. Due to the potential carcinogenic consequences of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), there is significant interest in natural antioxidants currently. *A. indica* may serve as an effective source of natural antioxidants¹¹.

The present investigation reveals an antioxidant activity *in vitro* demonstrated that the methanolic, n- butanolic and ethyl acetate extract of leaf of *A. indica* is highly efficient. Further investigation is required to ascertain the efficacy of this plant as an antioxidant in living organisms and to identify the specific components responsible for its actions. It may

have antioxidant activity *in vitro* by chelating metal ions and scavenging free radicals. Consequently, it may serve as a natural antioxidant.

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