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IN-VITRO ASSAY OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT AND LEAF DERIVED CALLUS EXTRACT OF ACALYPHA INDICA L.

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ABSTRACT

Acalypha indica (Indian Acalypha), commonly known as Mukhtajhuri in West Bengal is an important medicinal plant of India. The leaves are known to possess antimicrobial and antifungal properties. Leaf decoction is said to have anti-inflammatory properties. The present study shows the presence of antioxidant and antibacterial properties of Acalypha indica leaf extract and leaf-derived callus extract. Antioxidant activity was studied through DPPH assay. Whereas, in vitro antibacterial study of the extracts were carried out by adopting agar well diffusion technique using the pathogens Escherichia coli (E. coli), Klebsiella sp, Salmonella sp, Bacillus sp. After 24 hours of incubation maximum zone of inhibition was found against Bacillus sp. for leaf extract and Salmonella sp. for callus extract. These activities may be due to presence of flavonoids, phenolic compounds and other secondary metabolites present in these extracts.

KEY WORDS

Acalypha indica, Antibacterial, Callus extract, Leaf extract, Radical Scavenging Activity.

INTRODUCTION

Acalypha indica, a common weed in many parts of Asia including of India, Bangladesh, Pakistan, Sri Lanka as well as tropical Africa and South America [1] belongs to the family *Euphorbiaceae*. In Ayurveda, it is known as "Kuppi", "Muktavarchaa" or "Haritamanjari". Previous reports have also shown that *Acalypha indica (A. indica)* contains acalyphine which is used in the treatment of sore gums and to have a post-coital antifertility effect [2], anti-venom properties [3], and wound healing effects [4], antioxidant activity [5], anti-inflammatory effects [6], acaricidal effects [7], diuretic effects [8] and antimicrobial activity [9]. The roots of *Acalypha indica* is used as laxative and leaves for scabies and others cutaneous diseases [10]. The leaf sap is used to treat wounds [11] as well as eye and skin infections. Historically these plants have provided a good source of anti-infective agents in treatment of asthma [12] and pneumonia and so have increasingly gained importance during recent years. Moreover, antioxidant compounds [13] like phenolic acids, polyphenols and flavonoids [14] present in this plant extract, scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and inhibit the oxidative mechanisms that lead to degenerative diseases [15]. This weed also has antimicrobial property. Previous reports have shown flavonoids [16] (kaempferol glycosides mauritianin, clitorin, nicotiflorin and biorobin) and secondary

metabolites present in leaves and flowers of this medicinal plant can be efficiently used in treatment of diseases caused by antibiotics resistant strains of bacteria. For these reasons, various parts of this plant are utilized in production of valuable drugs to cure nosocomial infections of *Staphylococcus* sp [17]. Moreover, *in vitro* production of callus from somatic plant tissues can be helpful in future drugs production for infectious diseases. So, this has prompted us to investigate antioxidant and antibacterial activity of leaf and callus derived from the leaves of this plant.

MATERIALS AND METHOD

***Acalypha indica* used for sample preparation**

Figure 1: Collection of whole plant of *Acalypha indica from* Barisha in South Kolkata, West Bengal, India.

(i) Plant material:

The whole plant of *A.indica* **(Figure 1)** was collected from the adjoining areas of Barisha (22.47^{0}N, 88.31^{0}E), South Kolkata locality and was used in preparation of leaf extract, callus induction and preparation callus extract.

(ii) Callus Induction:

The leaf explants were washed with sterile water. Then the leaves were dipped in 1% sodium hypochlorite solution for 30 minutes, followed by rinsing twice with sterile water. The leaves were washed with 0.1% mercuric chloride solution for 30 seconds followed by rinsing twice with sterile water. Finally, the leaves were washed with fungicide Bavistin for 45 seconds followed by rinsing with sterile water.

(iii) Callus Production:

The surface sterilized leaves were trimmed and placed on Murashige and Skoog's (MS) medium (HiMedia, Mumbai) supplemented with 5µM 2,4-dichlorophenoxyaceticacid (2,4-D) and kept in culture rack at 22-25^{0}C temperature and alternative light for 16 hours and dark for 8 hours at Relative Humidity maintained at 60-70%. The complete formation of calli was obtained in 14 days and was allowed to propagate for 3 months **(Figure 2)** before taken for extraction.

Completely formed Callus

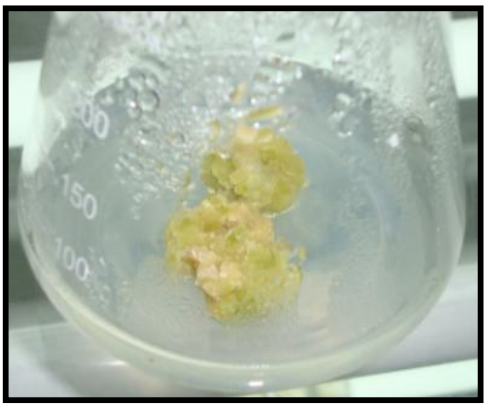

Figure 2: Induction of leaf-derived callus in MS medium supplemented with 5µM 2,4-D.

(iv) Preparation of leaf and callus extracts:

The air dried leaves were crushed with mortar and pestle and were extracted with methanol for three consecutive days at room temperature. The methanolic extract was evaporated to dryness and the crude residue left behind was weighed down. The residue was named as methanolic extract (LME).

The callus obtained was also air dried, crushed, extracted using methanol and filtered. The filtrate was evaporated and dissolved in methanol to obtain callus methanolic extract (CME).

(v) Determination of total phenolic content:

The extracts were dissolved in methanol to obtain a concentration of 1mg/ml. 100µl of these solutions were taken in a test tube and to it 100µl of 50% Folin Ciocalteau (Merck Specialist Pvt. Ltd) reagent was added. The mixture was then incubated for 3 minutes at room temperature and 2ml of 2% sodium carbonate solution was added. The volume was made up to 3ml with double distilled water [18]. The mixture was kept for 1 minute in water bath at 100^0C and allowed to cool in dark. The absorbance of the samples was noted at 720 nm using a UV-Vis spectrophotometer. The total phenolic content of LME and CME was calculated from standard curve of Gallic acid 1mg/ml.

(iv) Determination of total flavonoid:

To determine total flavonoid content, 150 µl of 5% $NaNO_2$ was added to 20 µl of LME and CME (1 mg/ml) and was incubated at room temperature for 5 minutes, followed by the addition of 2.5ml of 10% $AlCl_3$ solution and incubated further for 6 minutes at room temperature [19]. The absorbance of the samples were noted at 510 nm. The total flavonoids contents of LME and CME was calculated from standard curve of quercetin 1mg/ml.

(vi) Determination of Radical Scavenging Activity:

Antiradical activity was measured by a decrease in absorbance at 517 nm of DPPH (2,2-Diphenyl-1-Picrylhydrazyl) solution [20] brought about by plant extracts. In this assay DPPH acts as an indicator for "**Radical Scavenging Activity"** and changes its deep violet colour to colourless or pale yellow in presence of antioxidant and help us to determine **Radical Scavenging Activity (**RSC) of the substances. Therefore**,** to determine RSC of the extracts a stock solution of DPPH (0.12 mg/ml) was prepared in methanol and the samples mixture taken in the test tubes was of 2ml out of which LME and CME were present in varied amount (5µl, 10µl, 20µl) as per the concentration and rest was methanol (1995µl, 1990µl, 1980µl) .Then to these test tubes, 1ml of DPPH solution was added to achieve the final volume of 3ml and kept for 20 minutes incubation in dark. After 20 minutes of incubation in dark the absorbance was measured at 517 nm. Decrease in the absorbance of the DPPH solution indicates an increase of the DPPH antioxidant activity and percentage of Radical Scavenging Activity (% RSC) was calculated from the following equation:

$$\% \text{ RSC} = \frac{(Ao - As)}{Ao} \times 100$$

(A_o = DPPH solution without the sample, As= DPPH solution with the sample.)

(vii) Determination of Antibacterial Activity:

Antibacterial property of *A. indica* was determined using Kirby Bauer method. The antibacterial activity was studied by spreading 100 µl of 24 hours old culture of (*Bacillus* sp*., E. coli, Salmonella* sp. and *Klebsiella* sp.) on Mueller Hinton Agar (HiMedia,Mumbai) and 100 µl of LME and CME were loaded in the wells of these plates and their zone of inhibitions were calculated in millimeter (mm) after 24 hours of incubation at 37^0C and the zone of inhibitions were compared with methanol as a negative control [21].

RESULTS

1. Calculation of total phenolic and flavonoid content of extracts:

Total phenolic and flavonoid content of LME and CME are summarized in **Table1**. Total phenolic concentration was found out using Gallic acid (GAE) as standard. Total phenolic content for LME was found out to be 26.6 ± 2.5mg GAE/g of methanol extract. In the same way, total flavonid concentration was found out using Quercetin (QE) as standard. The total flavonoid content LME

was found to be 21.5 mg Quercetin/g of extract. Whereas, the total flavonoid content of CME was found to be 19.0 ± 3 mg/g of extract.

Table 1: Total phenolic and flavonoid content of extracts:

Sample	Total phenolic content (mg GAE± 2.5/g of extract)	Total flavonoid content (mg QE± 3/g of extract)
LME	26.60 ± 2.5	21.50 ± 3
CME	26.00 ± 2.5	19.0± 3

2. Calculation of Radical scavenging activity:

Percentage of RSC **(Table2)** was determined by DPPH assay at different concentrations for the extracts of which 250µg of LME showed maximum 43.57 % of RSC. Whereas, same concentration of CME had only 8.65% RSC.

Table 2: Percentage of Radical scavenging activity

Sample	Concentration (µg)	% RSC
LME	250	43.57
CME	250	8.65

3. Antibiotic sensitivity test:

Antibacterial test was done by measuring the diameter of the zone of inhibition **(Table 3)** by all the extracts on the test organisms and the antibacterial activity was classified [22] into the following types of test organism(s):

>12 mm zone of inhibition is **high sensitivity**

9-12 mm zone of inhibition is **moderate sensitivity**

6-9 mm zone of inhibition is **less sensitivity** and

< 6 mm zone of inhibition is **resistant**.

Thus, from the classification it was seen that *Bacillus* sp. was maximum sensitive to LME on with zone of inhibition 17±0.5 mm followed by *Salmonella* sp with diameter of zone of inhibition 15±0.5 mm. Whereas, *E. coli* has shown moderate sensitivity with diameter of zone of inhibition 10.06 ± 0.4mm in LME. The assay has also shown *Klebsiella* sp. was resistant to LME with diameter of zone of inhibition 3±0.2mm. This assay has also shown *Salmonella* sp. was less sensitive to CME with zone of inhibition of 8±0.2 mm. Whereas, *Klebsiella* sp., *E. coli* and *Bacillus* sp. was found to be resistant to CME with zone of inhibitions 2±0.3 mm,2±0.2 mm and 2.3±0.2 mm respectively.

Table 3: Antibiotic sensitivity of test organisms:

Test Organism	Zone of inhibition(mm)	
	LME	CME
Klebsiella sp.	3.3± 0.2	2.0± 0.3
E. coli	10.06± 0.4	2.0± 0.2
Bacillus sp.	17.00± 0.5	2.3± 0.2
Salmonella sp.	15.00± 0.5	8.0± 0.2

DISCUSSIONS

In recent times due to beneficial effect of *Acalypha indica* researchers have gained interest in studying the antioxidant activity of this type of medicinal plant extracts to treat degenerative diseases [23] because antioxidant compounds of these medicinal plants may acts as radical scavengers when added to the food products and prevent the radical chain reaction of oxidation and increase shelf life by retarding the processes of lipid peroxidation [24]. This antioxidant activity may be due to presence of phytochemicals like phenolics and flavonoid compounds. In this investigation we have also found that all the extracts have significant phenolic and flavonoid compounds in them. The result has also shown LME has maximum phenolic and flavonoid contents, compared to CME. So, due to presence of maximum phytochemical compounds, 250 µg of LME has shown 43.57% Radical scavenging activity and may be due to presence of less phytochemical compounds, 250 µg of CME has shown 8.65% of Radical scavenging activity. Previous reports have also stated that *Euphorbiaceae* [25] showed antimicrobial activity due to presence of high concentration of different compounds like flavonoids, phenols and alkaloids in it. This may be the reason in our investigation we have found out both extracts have antibacterial property against many common pathogens. Maximum antibacterial property is shown by LME against *Bacillus* sp. (zone of inhibition 17±0.5 mm). This extract has also shown highly sensitivity to *Salmonella* sp. (zone of inhibition 15±0.5mm), moderate sensitive to *E. coli* (zone of inhibition 10.06±0.4mm) and resistant to *Klebsiella* sp. (zone of inhibition 3.3±0.2 mm).Whereas on the other hand, CME has shown only less sensitivity to Gram negative *Salmonella* sp. (zone of inhibition 8±0.2 mm) and resistant to all other test microorganisms. From the different zone of inhibitions, we can conclude that the maximum antibacterial activity of LME may be due to the presence of maximum phenolic and flavonoid content in it. Our maximum antibacterial activity of LME towards Gram positive *Bacillus sp*. may be due to difference in cell wall compositions compared to Gram negative bacteria. This fact can be checked by employing more bacterial strains to find out the actual reason behind it. Moreover, various reports [26, 27] have already shown entrances of antibiotics through cell envelope (the outer and cytoplasmic membrane) are highly efficient for Gram positive bacteria depending on reaction with the protein layer (mucopolysaccharides or peptidoglycans). Moreover, the factors responsible for more sensitivity of the leaf extract towards bacteria are not exactly known but may be the presence of soluble secondary plant metabolites in LME. Hence, the present study showed both LME and CME have antioxidant and antibacterial property but LME has a potential to be a better antioxidant and antimicrobial agent in future.

CONCLUSION

This is first time a comparison is made on antioxidant and antibacterial activity of leaf extract (LME) and callus extract (CME). However, CME did not show any significant antioxidant and antibacterial activity even at high doses. So, further studies are required to increase the antioxidant and antibacterial activity of CME and identify the exact composition of the phenols, flavonoids and other secondary metabolites of both LME and CME.

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