Nat. Volatiles & Essent. Oils, 2021; 8(5): 5965 - 5977

Evaluation Of Antioxidant And Anti Inflammatory Activity Of Acalypha Indica Aqueous Ethanolic Extract

L. Akshayaa
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences
Saveetha University
Chennai - 77,
TamilNadu, India.
Email id :151801008.sdc@saveetha.com

Dr.Sankari Malaippan
Associate Professor ,
Department of Periodontics,
Saveetha Dental College and Hospitals ,
Saveetha Institute of Medical and Technical Sciences
Saveetha University
Chennai - 77,
TamilNadu, India.
Email id : sankari@saveetha.com

Dr. Rajeshkumar. S
Department of Pharmacology,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai -77
Tamil Nadu, India.
Email id : ssrajeshkumar@hotmail.com

Abstract

Background: *Acalypha indica* Linn is a natural herb, belonging to the family Europhorbiacaea that grows in tropical parts of India, Srilanka and throughout South America. It is considered as a traditional medicine of India for its wide medicinal properties. The extracts obtained from the root and leaf of *Acalypha indica* exhibit various phytochemical components like flavonoids, alkaloids, phenolic compounds and sterols, which are known to have good medicinal aspects on humans and animals.

Aim: The main objective of this study is to evaluate the antioxidant and anti-inflammatory activity of *Acalypha indica* aqueous ethanolic extract.

Materials and Methods: A coarse powder of Acalypha indica (1gm) was mixed with 25 ml of distilled water and ethanol in a 250 ml beaker. After 24 hrs, it was heated at 45-50 C for 10 minutes. Then the obtained aqueous ethanolic extract was stored in the refrigerator and further used to evaluate the antioxidant and anti-inflammatory effect using the DPPH method and albumin denaturation assay.

Results: The results demonstrated that both antioxidant and anti-inflammatory activity analysed using aqueous ethanolic extract of Acalypha exhibited a good inhibition rate against standard drugs Vitamin C and diclofenac.

Conclusion: Antioxidant and anti-inflammatory effects analysed using aqueous ethanolic extract of acalypha possess good medicinal properties. So it can be effectively formulated as herbal medicine that can be used to treat several diseases like skin disorders, wound infections, respiratory infections.
Keywords: *Acalypha indica*; aqueous ethanolic extract; antioxidant; anti inflammatory; innovative technique.

Introduction

Acalypha indica Linn is a natural herb belonging to the family Europhorbiacaea that grows in tropical parts of India, Srilanka and throughout South America. It is considered a traditional medicine of India for its wide medicinal properties (1). About 88% of the world's populations have turned to plant-derived drugs, as they are considered as the first-line defence for maintaining good health (2). Several plants have now become the major source of drugs used in treating many diseases. The plant *Acalypha indica* commonly used to treat cold, respiratory infections, skin infections, wounds, rheumatoid arthritis. It is also found to have good wound healing, antioxidant, anti-venom, antibacterial, anti-inflammatory properties (3). Besides this, acalypha plant can be used widely for external therapeutic approaches such as for constipation, headache, insect bites, epilepsy, dermatological ailments, gum and disease involving oral cavity (4).

Nanotechnology is one of the newest and most promising approaches in advanced medical science. Recently, there are many routes available for the synthesis of nanoparticles, as a rising need to promote low cost, non-toxic medicines produced in eco-friendly procedures. Green synthesis of the plant offers useful and compatible biomedical applications. In the present years, an advancement in the study of antioxidant activity from plant extracts has been grown. The extracts obtained from the root and leaf of *Acalypha indica* exhibit various phytochemical components like flavonoids, alkaloids, phenolic compounds and sterols, which are known to have good medicinal aspects on humans and animals. The phenolic substances of the plant that act as an effective antioxidant have been reported in several studies (5). The whole plant extract obtained from acalypha consists of a chemical compound called ‘acalyphine’ which is used externally to treat scabies and other skin diseases (6). Many studies have reported on the anti-inflammatory effect of different plants using nanoparticles. Scientific methods for synthesis of nanoparticles using advanced enzymes, plants, microbes or plant extracts are considered as possible adjuvant to chemical and physical methods. Many phytochemical studies on plants have inferred that aqueous ethanolic extracts of acalypha indica showed good wound healing effect, antioxidant, antimicrobial activities (7).

The antioxidants produced from the plant extracts act as free radicals, which are used to treat degenerative diseases (8,9),(10). As several studies illustrated the various pharmacology activities, it was highlighted the antioxidants from acalypha indica found to exhibit significant healing especially as an analgesic, anti-venom, hepatoprotective, and wound healing activities (11). In the earlier studies, it has been observed that administration of ethanolic extract of acalypha, found to significantly inhibit the venom-induced lethality and haemorrhage with a cardiotoxic effect (12). Nowadays, oxidative stress caused by free radicals in the human body leads to many degenerative diseases (13). Our team has extensive knowledge and research experience that has translated into high quality publications (14–

26),(27–31)(32)(33). Thus our present study has been aimed to evaluate the antioxidant and anti-inflammatory effect using aqueous ethanolic extracts of *Acalypha Indica.*

Materials and methods

Preparation of aqueous ethanolic plant extracts

The coarse powder of Acalypha indica (1gm) was mixed with 25 ml of distilled water and ethanol in a 250 ml beaker. Then the solution was kept in a shaker under room temperature for 24 hrs to remove the ethanol by evaporation. After 24 hrs, it was heated at 45- 50 C for 10 minutes. The mixture was then filtered in a conical flask using filter paper. Then the obtained aqueous ethanolic extract of the plant was stored in a refrigerator for further use. Therefore, the extract was then used to evaluate the antioxidant and anti-inflammatory activity using the DPPH method and albumin denaturation assay.

Figure 1: Prepared aqueous ethanolic extract of *Acalypha indica*

Inhibition of the antioxidant activity

DPPH Method

The antioxidant activity of aqueous ethanolic extract of plant was demonstrated using the DPPH method. About 1ml of DPPH solution in methanol and 450 microlitre of 50 mM Tris HCL buffer (ph 7.4) added to five test tubes, each labelled from 10 to 50μl. Different concentrations of plant extract and standard (Vitamin C) were added to each test tube. And the set-up was incubated in a dark room for 30 minutes. After incubation, the reduction in the quantity of DPPH free radicals was observed on the absorbance of the concentration at 517 nm.

Percentage of inhibition calculated using this formula:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample} \times 10}{\text{Absorbance of control}}$$

Inhibition of the anti-inflammatory activity

Albumin denaturation assay

The anti-inflammatory activity of aqueous ethanolic extract of the plant was demonstrated using the Albumin denaturation assay. About 0.45 ml of bovine serum albumin, 1% aqueous solution was added to five tubes. Different concentrations of plant extract and standard (Diclofenac sodium) were added to each test tube. The samples were incubated at room temperature for 20 minutes and heated under a water bath for 10 minutes. Then the samples were cooled, and the absorbance value was estimated under the spectrometer at 660 nm.

Percentage of protein denaturation was determined using this equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample} \times 10}{\text{Absorbance of control}}$$

Results

Figure 2: Evaluation of antioxidant effect using aqueous ethanolic extract by DPPH method, with test tubes labelled from 10 µl to 50 µl.

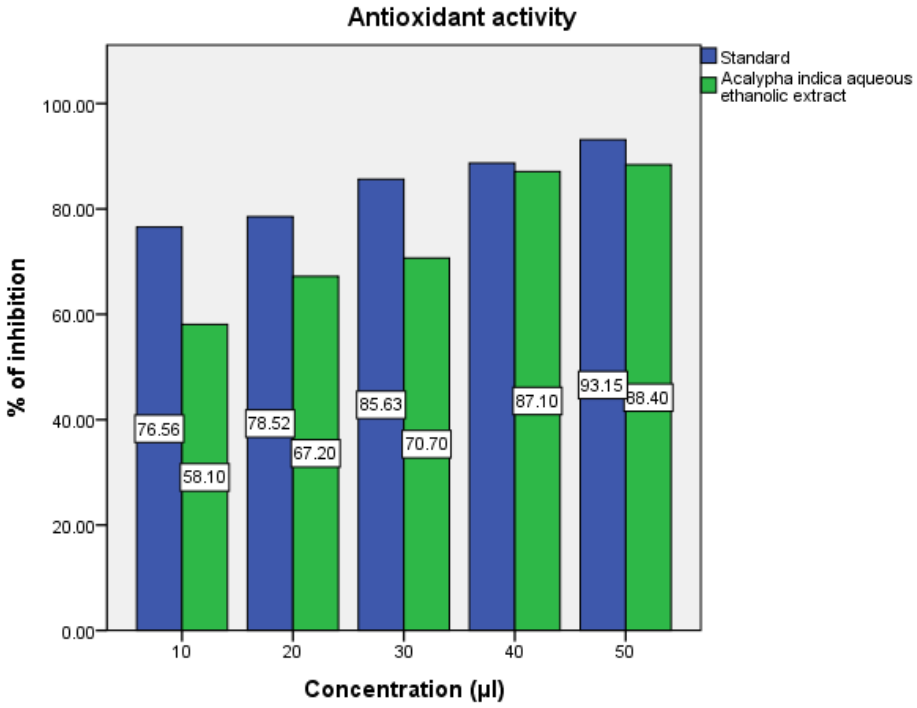

Figure 3: Bar graph represents the antioxidant activity of *Acalypha indica*. It represents an association between the concentration and percentage of inhibition shown by the standard and aqueous ethanolic extract of acalypha. X-axis represents the different concentration of standard and *Acalypha indica* extract added in microlitres (µl), Y-axis represents the percentage of inhibition shown by the concentration of the standard (Vitamin C) and Acalypha indica extract. Here, blue denotes the percentage of inhibition shown by standard and green denotes the percentage of inhibition shown by *Acalypha indica.* From the graph, it has been inferred that, at the maximum concentration of 50 µl, it shows 88% inhibition rate which is equal to the inhibition of standard drug.

Figure 4: Evaluation of anti-inflammatory effect using aqueous ethanolic extract by albumin denaturation assay, with test tubes labelled from 10 µl to 50 µl.

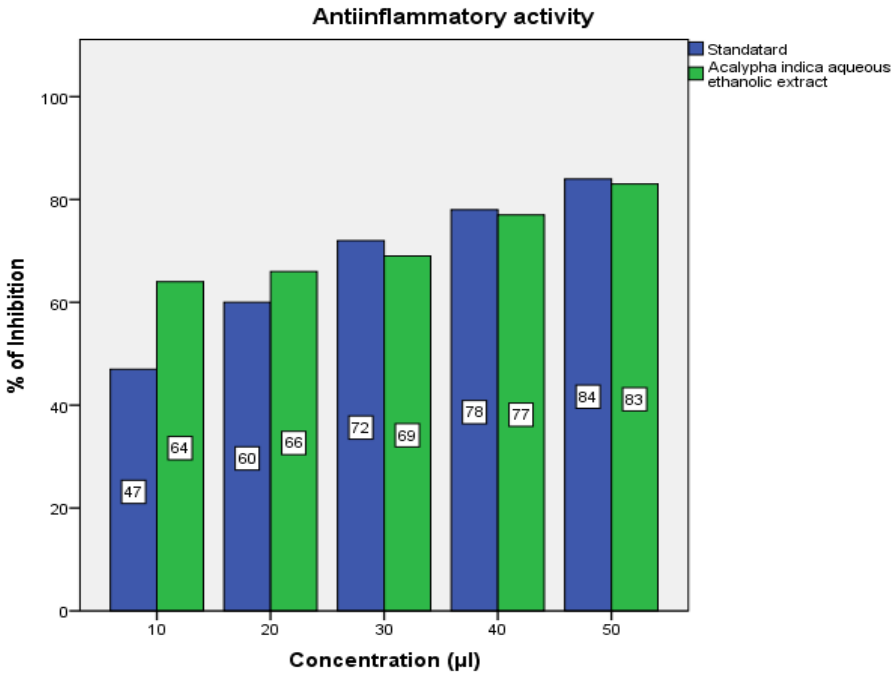

Figure 5 : Bar graph represents the anti-inflammatory activity of *Acalypha indica*. It represents an association between the concentration and percentage of inhibition shown by the standard and aqueous ethanolic extract of Acalypha indica. X axis represents the concentration of extract added in microlitre (µl), Y axis represents the percentage of inhibition shown by the concentration of standard (Diclofenac) and *Acalypha indica* extract. Here, blue denotes the percentage of inhibition shown by standard and green denotes the percentage of inhibition shown by *Acalypha indica.* From this graph, its has been confirmed that anti inflammatory activity of *Acalypha indica* aqueous ethanolic extract, at concentration of 50 µl, shows 83 % equipotent inhibition against the standard anti inflammatory drug.

Discussion

From the results obtained, we evaluated the antioxidant and anti-inflammatory effect of aqueous ethanolic extract of acalypha indica using the DPPH method and albumin denaturation assay. In figure 3, it was observed that the percentage of inhibition was highly effective by increasing the concentration of aqueous ethanolic extract. As it shows an equal inhibition to the standard antioxidant drug (Vitamin C). It has been inferred that when the concentration of ethanolic extract increased to 50 µl, there is 88 % significant inhibition rate found which is equal to the inhibition of standard drugs. In figure 5, the aqueous ethanolic extracts of *Acalypha indica e*xhibited a good anti-inflammatory effect which was estimated under albumin denaturation assay. It seems to show an equipotent effect as the standard drug, diclofenac. From the results obtained, it has been interpreted that the anti-inflammatory effect of aqueous ethanolic extract of acalypha, at a concentration of 50 µl, shows 83 % equipotent inhibition against the standard anti-inflammatory drug diclofenac.

In our present study, we found that by increasing the concentration of plant extract, it shows a good inhibitory effect against the standard drugs. A similar study done by Madhavan et al, illustrated that antioxidant activity of acalypha found by increasing concentration of the different plant extracts shows highest free radical scavenging (34). Another study demonstrated that methanolic extract of *acalypha* has shown better antioxidant potential when compared to standard ascorbic acid by DPPH scavenging assay method (35). Many studies have also reported that antioxidant effects of *Acalypha indica* extract were used to treat many degenerative diseases. As these plants contain many antioxidant properties which act as radical scavengers, so when added to food it prevents the free radical chain reaction and also will increase shelf life by retarding lipid peroxidation in cells . A recent study has illustrated the good antioxidant effect is due to the phytochemical content like phenolic and flavonoid compounds. So this reveals that the maximum phenolic compounds in the plant show good antioxidant property (36),(37).

A previous study, which was done using acetone and ethanolic extracts of plants at different concentrations such as 50, 100, 250, 500 µg/ml showed effective antioxidant activity in a concentration dependent manner (38). Likewise a similar study has revealed that plant extracts showed significant antimicrobial and antioxidant activities in relation to the presence of tannins, flavonoids and other alkaloids. Maximum rate of phytochemicals was observed in acetone leaf extracts when compared to petroleum ether, chloroform, ethyl acetate and methanolic extracts of acalypha (39). From the results obtained, it has been found that aqueous ethanolic extracts of the plants showed significant inhibitory effect on both anti-inflammatory and antioxidant properties . A similar study done by Ngibad K et al, reported that ethanolic extracts of acalypha at higher concentrations have been found to capture more free radicals with increase in IC 50 value in DPPH method (40). A previous study has compared the antioxidant and antimicrobial property using callus derived leaf extract and methanolic leaf extract of acalypha. It has been observed that a high percentage of free radical scavenging activity was observed in methanolic extract when compared to callus derived extracts (41).

In our study, the aqueous ethanolic extracts of *Acalypha indica* exhibited a good anti-inflammatory effect which was estimated under albumin denaturation assay. It seems to show an equipotent effect as the standard drug, diclofenac. An earlier study, done using the ethanolic leaf extracts of acalypha, found to have a significant effect, as it shows a maximum percentage of inhibition of about 85% when compared

to standard drug ibuprofen . Several studies have inferred that the methanolic extract of *A. indica* L found to have a good anti-inflammatory effect in a dose-dependent manner (42). An experimental study done by Suresh Reddy et al, reported wound healing effects of leaf extracts of acalypha in rats by topical application. The results of this study has been inferred that 10 % leaf extracts of Acalypha indica being prepared with saline showed better wound healing activity with formation of low collagen. An in vivo study done using the ethanolic extract of Acalypha indica was tested in healing of burns in rabbits. Ethanolic extract was formulated as ointment in rabbits, and it showed good wound healing effect within 14 days (43). Nevertheless, the assorted pharmacological activities of *Acalypha indica* isolated have been examined in various experimental studies but its outcomes may not really be convenient to the circumstance in humans. Several pharmacological investigations directed on *Acalypha indica* demonstrate the massive capability of this plant to be used to treat wounds, coughs, inflammatory conditions and diabetes.

Conclusion

Thus our present study has concluded that antioxidant and anti-inflammatory activity of aqueous ethanolic extract of acalypha indica, at maximum concentration of 50 µl, shows equal inhibition rate to standard drugs Vitamin C and diclofenac sodium. Therefore our future scope in this study needs to evaluate the antimicrobial and antifungal properties using aqueous ethanolic extract of acalypha indica which can be utilised to formulate newer mouthwashes, periodontal dressings for the treatment of periodontal diseases.

Legends

Figure 1: Prepared aqueous ethanolic extract of *Acalypha indica*

Figure 2: Evaluation of antioxidant effect using aqueous ethanolic extract by DPPH method, with test tubes labelled from 10 µl to 50 µl.

Figure 3: Bar graph represents the antioxidant activity of *Acalypha indica*. It represents an association between the concentration and percentage of inhibition shown by the standard and aqueous ethanolic extract of acalypha. X-axis represents the different concentration of standard and *Acalypha indica* extract added in microlitres (µl), Y-axis represents the percentage of inhibition shown by the concentration of the standard (Vitamin C) and Acalypha indica extract. Here, blue denotes the percentage of inhibition shown by standard and green denotes the percentage of inhibition shown by *Acalypha indica.* From the graph, it has been inferred that, at the maximum concentration of 50 µl, it shows 88% inhibition rate which is equal to the inhibition of standard drugs.

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Acknowledgement

The authors are thankful to the Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical science, Saveetha University for providing a platform in expressing their knowledge.

Conflict of interest

The authors declare no conflict of interest.

Source of funding

The present project is sponsored by the following agencies

- Saveetha Dental College,
- Saveetha Institute of Medical and Technical science,
- Saveetha University,
- Lingam stores (LTSN/7233).

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