Preliminary Phytochemical Analysis and Assessment of Antioxidant and Anti Cholesterol Activity of Ethanolic Leaf Extract of Acalypha indica linn

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Acalypha indica Linn. is a weed plant found in shady wetlands. It is commonly called as kuppai meni in Tamil, belonging to the family Euphorbiaceae. It is found to be one of the greatest Indian plants with anti-venom, antibacterial, antiviral and antimicrobial properties.

Aim: Preliminary phytochemical analysis and assessment of antioxidant and anti cholesterol activity of ethanolic leaf extract of acalypha indica linn

Materials and Methods: Phytochemical screening and in vitro antioxidant and hypolipidemic potential of Acalypha indica Linn. was analysed as per the standard methods. The data were analyzed statistically by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test and it was used to see the statistical significance among the groups. The results with the p<0.05 level were considered to be statistically significant.

Results: Ethanolic leaf extract of Acalypha indica Linn exhibited a significant antioxidant and
hypolipidemic activity and increased in a dose dependent manner as compared to the standard drug.

Conclusion: Many researchers are working towards replacing synthetic drugs with herbal alternatives. Herbal extracts are natural and will aid in longevity.

Keywords: Acalypha indica; weed plant; standard drugs; In-vitro analysis of antioxidant and anti-cholesterol; innovative technology; novel method.

1. INTRODUCTION

Acalypha indica Linn. is a weed plant found in shady wetlands. It is commonly called as kuppai meni in tamil, belonging to the family Euphorbiaceae. It is found to be one of the greatest Indian plants with anti-venom, antibacterial, antiviral and antimicrobial properties [1]. Indian medicine like Ayurveda, siddha, utilize the extract to treat diseases like Pneumonia, asthma, wounds, allergies, cleansing liver and kidney etc. As a folk medicine, the extract is used as facial masks and other utilities [2]. It is considered as one of the greatest gifts of mother earth.

This Plant produces a wide range of bioactive molecules which result in a cure to a wide range of diseases. The most important phytoconstituent of the extract are alkaloid, flavonoid, tannin and Phenolic compounds [3]. These compounds are found in several parts of plants like root, shoot, leaf and bark. These phytochemicals were analysed and studied by many researchers in many parts of the world (Pauranik et al. 1986) [4,5] has predicted that the variety of biomolecules produced by the plant can act as an antimicrobial and antioxidant agent which constitute 80% of most popular and healthy natural remedies.

This plant has been reported for various clinical constituents like kaempferol glucoside, clitorin, mauritian, nicotiflorin and borodin. Which were isolated and few recent studies of flowers and leaves [6,7].

Hyperlipidemia is a major risk factor causing cardiovascular disease which can result in cardiac arrest; it is responsible for the cardiac risks associated with one third of the population all over the globe. It is caused by the over deposition of cholesterol and triglyceride, which may have resulted due to the unhealthy food practice which has resulted in the production of free radicals that cause fast aging and decreased physical flexibility [8,9].

Generally many synthetic drugs are used as a hypolipidemic agent. Synthetic drugs generally inhibit HMG CoA reductase, a key enzyme in cholesterol biosynthesis. Simvastatin is the most commonly used HMG CoA reductase inhibitor [10]. Simvastatin is also used as a blood thinner. It is used to reduce bad cholesterol like LDL, triglyceride and raise good cholesterol in blood. It belongs to the group of drugs 'statin'. It reduces the fats produced by the liver. As a result it reduces the effect of cardiac disease. However this drug has some side effects like headache, constipation, muscle spasms, rigidity, etc.

Antioxidants are compounds which fight against free radicals. Increased production of free radicals due to increasing pollution and lifestyle problems, tend to age faster and may also lead to cancer and other degenerative disease [11].

Humans are constantly getting exposed to reactive oxygen radicals which are generated by various biotic and abiotic factors like irradiation, pollutants,stress, etc. These free radical attack proteins, DNA, enzymes and cause tissue degeneration leading to many effects like ageing, atherosclerosis, heart disease, etc [12,11]. One of the important free radicals we encounter is oxygen radical, which causes most of these degenerative diseases. It takes the electron present in DNA, proteins, etc. Cause breakage of bonds.

Antioxidants are compounds obtained naturally or manufactured in a lab which can scavenge these free radicals and stop free radical chain reactions which can increase lifespan. The standard drug used as antioxidants currently are ascorbic acid or Vitamin C. It donates free electrons to the oxygen radical and scavenges them from damaging the cells. The activity of tannin, phenolic acid and flavonoids showed a great response in antioxidant reaction [13,14]. Various research on herbal extracts are also taking place in industries and pharmaceutical companies to explore their antimicrobial and antioxidant properties [15-35]. In the current
study in vitro antioxidant and hypolipidemic potential of Acalypha Indica linn. was analysed.

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

2.1.1 Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

2.1.2 Test for carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

2.1.3 Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

2.1.4 Test for alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

2.1.5 Test for terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H₂SO₄ was added. Red color ppt obtained indicates the presence of terpenoids.

2.1.6 Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

2.1.7 Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

2.1.8 Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2.2 DPPH free Radical Scavenging Activity of Acalypha indica

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0.5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control OD} - \text{Sample OD} \times 100}{\text{Control OD}}$$

2.3 In vitro Anti-cholesterol Activity of Acalypha indica

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg/mL. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000 μL of R1 reagent and 10 μL of cholesterol as sample. Twenty microliter of distilled water and 2000 μL of R1 reagent were used as blank. Negative control consisted of 20 μL cholesterol and 2ml R1; standard consisted of 20 μL simvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control} - \text{Sample} \times 100}{\text{Negative control}}$$

2.4 Statistical Analysis

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA)
and Duncan’s multiple range test to assess the significance of individual variations between the groups. In Duncan’s test, significance was considered at the level of p<0.05.

3. RESULTS AND DISCUSSION

The result of qualitative phytochemical analysis of *Acalypha indica* leaf extracts are given in table 1. The result reveals a strong presence of flavonoid, alkaloids, saponins, steroids and amino acids. The DPPH radical scavenging activity revealed the antioxidant potential of the plant extract. Antioxidant potential was significantly exhibited by the *Acalypha indica* extract with IC50 = 290 ug/ml and increased in a dose dependent manner as compared to the standard drug Vitamin C (IC50 of Vitamin-C= 210 ug/ml) (Fig. 1).

The result also revealed a strong in vitro anti cholesterol potential of ethanolic extract of *Acalypha Indica*, which is slightly lower than that of standard simvastatin. IC50 of *Acalypha indica* was found to be 300ug/ml and the standard drug simvastatin exhibited IC50 at 260ug/ml. (Fig. 2).

Previous studies strongly suggest that the plant extracts which are rich in phenolic compounds can fight free radicals effectively, since aromatic rings in phenolic compounds can easily accept or donate an electron (Jennings, 1981).

Table 1. Phytochemical screening of *Acalypha indica*

| Phytochemicals | Leaf extract |
|----------------|--------------|
| Proteins       | (-)          |
| Amino acids    | (+)(+)(+)    |
| Terpenoids     | (+)(+)       |
| Flavonoids     | (+)          |
| Alkaloids      | (+)          |
| Saponins       | (+)          |
| Steroids       | (+)          |

Thus, in the present study, the plant extract was found to be rich in phenolic compounds such as alkaloids and flavonoids, which forms the basis for its strong antioxidant property.

Cholesterol is a steroid which is produced endogenously and also taken from the diet. The biosynthesis of cholesterol is usually regulated by regulating the key enzyme HMG CoA reductase [36]. Standard anti cholesterol drugs act by inhibiting these key enzymes. Though the standard drugs are more potent in reducing cholesterol levels, it also exhibits various side effects if consumed for a longer period of time [37].

![DPPH radical scavenging activity](image)

Fig. 1. The graph depicts the association between In vitro antioxidant activity of *Acalypha indica* and standard drug simvastatin( vitamin C). X-axis represents activity of drugs and Y-axis percentage of DPPH scavenging activity. Blue represents activity of std drug simvastatin and orange represents activity of *Acalypha indica*. The graph denotes that standard drug simvastatin shows greater activity than that of ethanolic extract of *Acalypha indica*. Each line Represents Mean ± SEM of 3 independent observations. Significance at p ≤ 0.05

*Wu et al., [16]*
FIGURE 2: The graph depicts the association between in vitro antioxidant activity of *Acalypha indica* and standard drug simvastatin (vitamin C). X-axis represents activity of drugs and Y-axis percentage cholesterol scavenged. Blue represents activity of std drug *Acalypha indica* and brown represents activity of simvastatin. The graph denotes that standard drug simvastatin shows greater activity than that of ethanolic extract of *Acalypha indica*. Each line represents Mean ± SEM of 3 independent observations. Significance at p ≤ 0.05

The study showed that the in vitro antioxidant property *Acalypha indica* extract is Ic50 = 290 ug/ml which is similar with [38,39,40]. The study also found that in vitro anti cholesterol potential of ethanolic extract of *Acalypha Indica*, which is slightly lower than that of standard simvastatin i.e. Ic50 of *Acalypha indica*, which is found to be similar with [41-42]. From the study, it was evident that the plant extract exhibited a strong anti cholesterol property. Further research needs to be done to explore various medicinal properties of the extract.

4. CONCLUSION

The ethanolic extract of *Acalypha indica Linn.* possessed potent antioxidant and anticholesterol activity. Which will be natural and may be with or without any side effects of std drug simvastatin. Nowadays people are moving towards the use of herbal products, as they are feeling fear about the side effects it may cause. So further research is needed to convert the extract into active drug formulation.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gupta RK. Medicinal & Aromatic Plants: With Colour Plates (hb); 2010.
2. Durai Pandian J, et al. Stroke and thrombolysis in developing countries,’
13. Young IS, McEneny J. Lipoprotein oxidation and atherosclerosis’, Biochemical Society transactions. 2001;29(Pt 2):358–362.

14. Liu YW, et al. A test of screening to predict lung cancer among dust-exposed tin miners with sputum imaging cytometry?’, Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2008;26(4):203–207.

15. Eyob S, et al. Traditional medicinal uses and essential oil composition of leaves and rhizomes of korarima (Aframomum corrorima (Braun) P.C.M. Jansen) from southern Ethiopia?’, South African Journal of Botany. 2008;181–185.

DOI: 10.1016/j.sajb.2007.10.007.

16. Wu F, et al. Biologically synthesized green gold nanoparticles from Siberian ginseng induce growth-inhibitory effect on melanoma cells (B16), Artificial cells, nanomedicine, and biotechnology. 2019;47(1):3297–3305.

17. Chen F, et al. 6-shogaol, a active constituents of ginger prevents UVB radiation mediated inflammation and oxidative stress through modulating NrF2 signaling in human epidermal keratinocytes (HaCaT cells). Journal of photochemistry and photobiology. B, Biology. 2019;197:111518.

18. Li Z, et al. Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanoparticles on rat glioma C6 cells via alleviating the MMP signaling pathway’, Journal of Photochemistry and Photobiology B: Biology. 2020;111773.

DOI: 10.1016/j.jphotobiol.2019.111773.

19. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management’, Biomedicine & pharmacotherapy = Biomedecine & pharmacotheraphy. 2020;131:110702.

20. Malaikolundhan H, et al. Anticarcinogenic effect of gold nanoparticles synthesized from Albizia lebbeck on HCT-116 colon cancer cell lines’, Artificial cells, nanomedicine, and biotechnology. 2020;48 (1):1206–1213.

21. Han X, et al. Anticarcinogenic potential of gold nanoparticles synthesized from Trichosanthes kirilowii in colon cancer cells through the induction of apoptotic pathway’, Artificial cells, nanomedicine,
and biotechnology. 2019;47(1):3577–3584.
22. Gothai S, et al. Pharmacological insights into antioxidants against colorectal cancer: A detailed review of the possible mechanisms', Biomedicine & pharmacotherapy Biomedicine & pharmacotherapy. 2018;107:1514–1522.
23. Veeraraghavan VP, Hussain S, et al. A Comprehensive and Critical Review on Ethnopharmacological Importance of Desert Truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea', Food Reviews International. 2021;1–20. DOI: 10.1080/87559129.2021.1889581.
24. Veeraraghavan VP, Periadurai ND, et al. Green synthesis of silver nanoparticles from aqueous extract of Scutellaria barbata and coating on the cotton fabric for antimicrobial applications and wound healing activity in fibroblast cells (L929)', Saudi journal of biological sciences 2021;28(7):3633–3640.
25. Sathy S, et al. An in vitro study on hexavalent chromium [Cr(VI)] remediation using iron oxide nanoparticles based beads', Environmental Nanotechnology, Monitoring & Management. 2020;14:100333.
26. Yang Z, et al. Piperine loaded zinc oxide nanocomposite inhibits the PI3K/AKT/mTOR signaling pathway via attenuating the development of gastric carcinoma: In vitroandin vivo studies', Arabian Journal of Chemistry. 2020;13(5):5493–5516.
27. Rajendran P, et al. Consumption of reused vegetable oil intensifies BRCA1 mutations', Critical reviews in food science and nutrition. 2020;1–8.
28. Barma MD, et al. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish', Archives of oral biology. 2021;126:105132.
29. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life?', International journal of paedodontic dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children. 2021;31(2):285–286.
30. Samuel SR, et al. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19', International journal of paedodontic dentistry / the British Paedodontic Society [and] the International Association of Dentistry for Children. 2021;31(3):436–441.
31. Tang Y, et al. Osteogenic differentiation and mineralization potential of zinc oxide nanoparticles from Scutellaria baicalensis on human osteoblast-like MG-63 cells', Materials Science and Engineering: C. 2021;111656. DOI: 10.1016/j.msec.2020.111656.
32. Yin Z, et al. Potential chemotherapeutic effect of betalain against human non-small cell lung cancer through PI3K/Akt/mTOR signaling pathway', Environmental toxicology. 2021;36(6):1011–1020.
33. Mickymaray S, et al. Rhaponticin suppresses osteosarcoma through the inhibition of PI3K-Akt-mTOR pathway', Saudi journal of biological sciences. 2021;28(7):3641–3649.
34. Teja KV, Ramesh S. Is a filled lateral canal – A sign of superiority? Journal of Dental Sciences. 2020;562–563. DOI: 10.1016/j.jds.2020.02.009.
35. Theertha M, et al. Innate lymphoid cells: Potent early mediators of the host immune response during sepsis', Cellular & molecular immunology. 2020;17(10):1114–1116.
36. Nagpal ML. Cholesterol: Good, Bad and the Heart. BoD – Books on Demand; 2018.
37. Huang C, Freter C. Cholesterol Lowering Therapies and Drugs. BoD – Books on Demand; 2016.
38. Reddy TP, Reddy Prasad Reddy T. Exploring the Anti-inflammatory and Anti-cancer compounds from the leaves of Acalypha indica', IOSR Journal of Pharmacy and Biological Sciences. 2012;1–7. DOI: 10.9790/3008-0420107.
39. Dwijayanti A, Farida S, Purwaningsih EH. Comparing Anti Aging Potential between Centella asiatica and Acalypha indica: Focus on Forelimb Muscle Strength', Advanced Science Letters. 2018;6058–6060. DOI: 10.1166/asl.2018.12621.
40. Zulkifli C, et al. Anti-Oxidative Potential of Acalypha indica L. Root Extract on Brain-Derived Neurotrophic Factor Levels in Old Sprague-Dawley Rats. Proceedings of BROMO Conference; 2018. DOI: 10.5220/0008357400580061.
41. Jayaprakasam RJR, Ravi TK. Evaluation of anti Arthritic activity of the root extract of acalypha indica linn. Using in vitro techniques, International Journal of Phytopharmacy; 2013.

DOI: 10.7439/ijpp.v2i6.36.

42. El-Shemy H. Aromatic and Medicinal Plants: Back to Nature. BoD – Books on Demand; 2017.

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