Original Research Article

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ASSESSMENT OF ANTIOXIDANT AND ANTI CHOLESTEROL ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *ACALYPHA INDICA LINN.*

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.

Running title: Antioxidant and anti cholesterol properties of *Acalypha indica Linn.*

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(Gupta, 2010). 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 (Durai Pandian *et al.*, 2007). 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 (Awadh *et al.*, 2017). 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)(Rai, Zachariah and Shrivastava, 1989).(Catlow, 1848), 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 (Shima, Hisada and Inagaki, 1972). (Doughari, 2007)

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(Nelson, 2013)3;(Ohlsen and Gaughran, 2011).

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(Ide *et al.*, 1990). 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.(Willcox *et al.*, 2004)

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.(Halliwell, 1994; Willcox *et al.*, 2004). 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.(Young and McEneny, 2001; Liu *et al.*, 2008). Various research on herbal extracts are also taking place in industries and pharmaceutical companies to explore their antimicrobial and antioxidant properties.(Eyob *et al.*, 2008). (Wu *et al.*, 2019),(Chen *et al.*, 2019),(Li *et al.*, 2020),(Babu and Jayaraman, 2020),(Malaikolundhan *et al.*, 2020),(Han *et al.*, 2019),(Gothai *et al.*, 2018),(Veeraraghavan, Hussain, *et al.*, 2021),(Sathya *et al.*, 2020),(Yang *et al.*, 2020),(Rajendran *et al.*, 2020),(Barma *et al.*, 2021),(Samuel, 2021),(Samuel *et al.*, 2021),(Tang *et al.*, 2021),(Yin *et al.*, 2021),(Veeraraghavan, Periadurai, *et al.*, 2021),(Mickymaray *et al.*, 2021),(Teja and Ramesh, 2020),(Theertha *et al.*, 2020)(Veeraraghavan, Periadurai, *et al.*, 2021). In the current study *in vitro* antioxidant and hypolipidemic potential of *Acalypha Indica linn*. was analysed.

Materials and Methods:

1. Phytochemical Screening test

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.

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.

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.

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.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

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.

Detection of saponins

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

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. 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:

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

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/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:

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

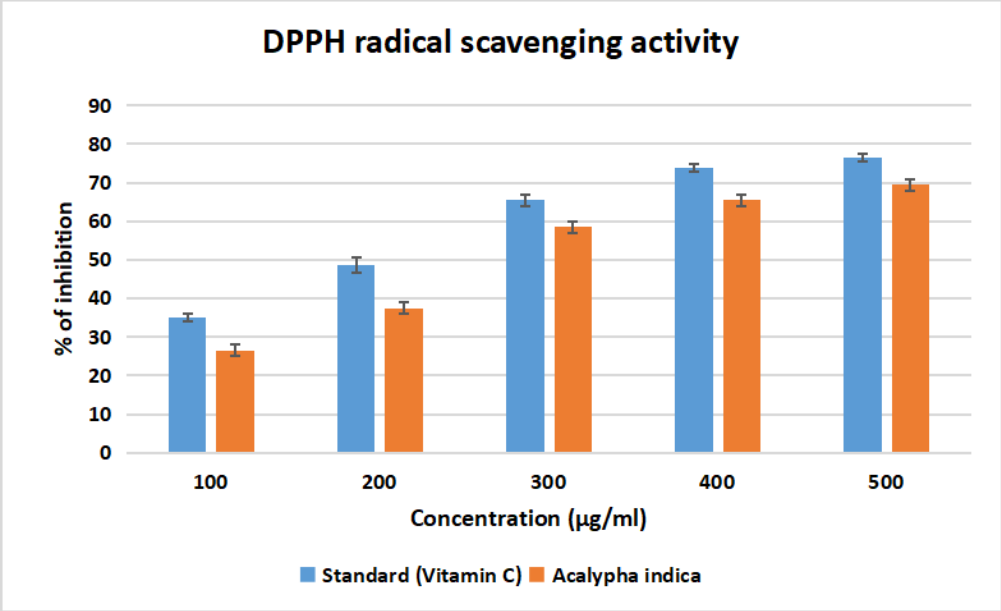
Statistical Analysis

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 $p<0.05$.

Results and Discussion:

Table 1: Phytochemical screening of *Acalypha indica*

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



(Wu *et al.*, 2019)

FIGURE 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 $p \leq 0.05$.

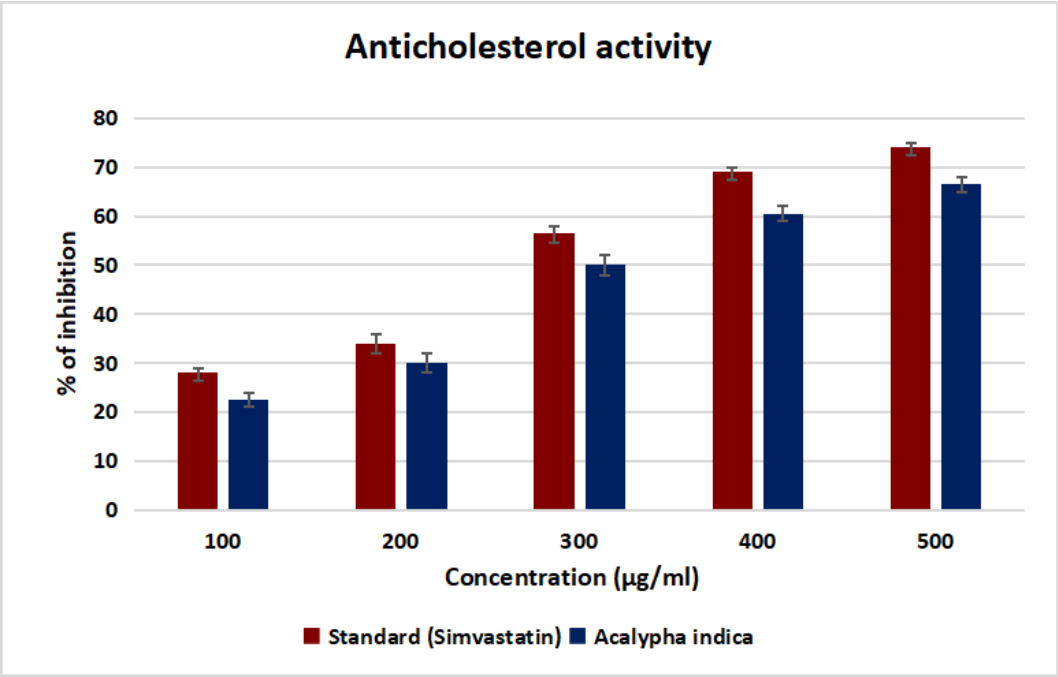


FIGURE 2: The graph depicts the association between In vitro antioxidant activity of *Acalypha indica* and standard drug simvastatin(vitamin C). X-axis represents activity of drugs and Y-axis percentage cholesterol scavenged. Blue represents activity of std drug *Acalypha indica* and brown represents 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 $p \leq 0.05$.

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)(Figure 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. (Figure 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)

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(Nagpal, 2018). 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(Huang and Freter, 2016).

The study showed that the in vitro antioxidant property *Acalypha indica* extract is Ic50 = 290 ug/ml which is similar with (Reddy and Reddy Prasad Reddy, 2012; Dwijayanti, Farida and Purwaningsih, 2018; Zulkifli *et al.*, 2018). 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 (R, Jayaprakasam and Ravi, 2013)(El-Shemy, 2017). 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.

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "herbal", "Ayurveda", "siddha" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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