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Evaluation of Anti hemolytic activity, antibacterial activity and phytochemical investigation of *Acalypha indica* methanolic leaf extract

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Abstract
The aim of the current study was to assess *Acalypha indica* methanolic leaf extract for its anti hemolytic activity, anti-bacterial activity and its various phytochemical constituents. The methanolic leaf extract of *Acalypha indica* shown to have reasonable anti hemolytic activity. The methanolic extract showed antihemolytic activity in the range from 27% to 54%. At varying concentration of plant extracts 20, 40, 60, 80 and 100 mg /ml the percent of inhibition of hemolysis recorded were 27%, 34%, 40%, 47%, 54% respectively. The methanolic leaf extract of *Acalypha indica* showed significant inhibitory effect on all of the five bacterial species such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Streptococcus mutans*. The inhibition zones for *S. aureus* were 14 mm, 16 mm and 20 mm (including well 4 mm) at concentrations of 15 µl, 20 µl and 25 µl respectively. The inhibition zones *P. aeruginosa* were 14 mm, 14 mm and 16 mm, 10 mm, 11 mm and 20 mm for *Streptococcus mutans*. The zones for *E. coli* were 10 mm, 12 mm and 21 mm and 14 mm, 16 mm and 19 mm for *Bacillus subtilis*. The phytochemical investigation of methanolic extract of *Acalypha indica* leaves showed the presence of carbohydrates, flavanoids, alkaloids, phenols and saponins.

Keywords: *Acalypha Indica*, Hemolysis, Phytochemical, Bacteria

Introduction
Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha [1]. Medicinal plants gaining lot of importance now a days because of efficacy they have been showing in the traditional healing [2]. Herbs are the source of magnificent inhibitors that could act on wide variety of diseases. One of the great aspect of herbs is they show 100% results when comes to the healing. Herbs have all sorts of answers against various diseases [3]. The best source of drugs without lethal effects to human systems could be the plant source and this has been proved by the Traditional healing system and the recent studies conducted on the experimental animals [4]. *Acalypha indica* (Euphorbiaceae) is a weed that has high medicinal value. In Ayurveda, it is known as "Kuppi", "Muktavarchaa" or "Haritamanjari" [5]. *Acalypha indica* possess various medicinal activities. *Acalypha indica* known to have wound healing effects [6]. Mohana *et al* (2008) [7] proved Acalypha indica has inflammatory effect. Singh *et al* (2004) [8] proved Acarcidal Property of Kuppaimemeni (*Acalypha indica*) against natural Psoroptes cuniculi infestion in broiler Rabbits. R. Shanmugapriya (2011) [9] reported that *Acalypha indica* has Antioxidant Potential and Antibacterial Activity. Shirwaikar *et al* (2004) [10] evaluated the neutralization potential of *Viper russelli* russelli (Russell's viper) venom by ethanol leaf extract of *Acalypha indica*. In this research study an attempt has been made to assess the phytochemicals present in leaf extract and its anti-bacterial and anti hemolytic activity against H_2O_2 induced hemolysis in RBC.

Materials and Methods
Materials
Leaves of *Acalypha indica* were collected from village Seshammachruvu, Achanta mandalam, West Godavari district. The plant was authenticated by Dr. Suryanayana Raju, Department of Botany, S V K P & Dr. K S Raju Arts and Science College, Penugonda. Leaves were dried in sunlight for a week and then powdered using blender to get coarse powder. Chicken blood was collected from the local market in EDTA bottle.

Fig 1: *Acalypha indica*

Test Microorganisms
The methanolic leaf extract of *Acalypha indica* were tested against five pathogenic bacteria. The test organisms include *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Streptococcus mutans.*

Extraction process
Preparation of extract was done according to the procedure done by Sharmila *et al*., (2011) [11]. 25g of Leaf powder was packed in soxhlet extraction unit and exhaustively extracted using 100ml of methanol at 60 ºC for 12 hours. The extract was completely dried in water bath at 40 ºC and subsequent stored at 4 ºC

Phytochemical analysis
A preliminary phytochemical investigation was conducted for the detection of steroids, terpenoids, flavonoids, saponins, tannins, carbohydrates, and phenols by using standard procedures [12, 13].

Determination of antibacterial activity
Antibacterial activity was measured by well diffusion method [14]. Nutrient agar (Hi media) was prepared and poured in to the petriplates. After solidification of media, overnight bacterial cultures were inoculated on the surface of media. By using a sterile gel puncher 4 mm of wells were made in each petri plates. Then 15 µl, 20 µl, 25 µl of methanolic leaf extract *Acalypha indica* were added in to the three wells respectively. The plates were incubated in the incubator at 37 °C for optimum bacterial growth. In the next day, diameter of the zone of inhibition was measured.

Assessment of Anti-hemolytic Activity
Anti hemolytic activity against H_2O_2 induced hemolysis in chicken RBC determined by in vitro method described by Tavazzi *et al*., (2001) [15] and Thagriki Dluya., (2015) [16]. The chicken erythrocytes were separated by centrifugation at 200 rpm and washed with saline or isotonic sodium phosphate buffer (pH 7.4) until the supernatant is colourless. The erythrocytes were then diluted with saline or phosphate buffer to give a 4% suspension. Varying amounts of the plant extracts (20, 40, 60, 80 and 100 mg /ml) with saline or buffer was added to 2 ml of the suspension of erythrocytes and the volume was made up to 3.5 ml with saline or buffer. This mixture was pre-incubated for 120 min and then 0.5 ml H_2O_2 solutions of appropriate concentration in saline or buffer was added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring 90% hemolysis of blood cells after 120 min incubation. After 120 min of incubation tubes were centrifuged and the amount of hemolysis was determined by measurement of the absorbance at 540 nm corresponding to hemoglobin liberation. Anti-hemolytic activity was expressed as the inhibition percentage and was calculated using the following formula:
Anti-hemolytic activity (%)

$$= \frac{\text{Control 540 nm} - \text{Sample 540 nm}}{\text{Control 540 nm}} \times 100$$

Where, Sample540 nm was the absorbance of the sample andControl540 nm was the absorbance of the control.

Results
Phytochemical analysis
The phytochemical investigation of methanolic extract of *Acalypha indica* leaves showed the presence of carbohydrates, flavanoids, alkaloids, phenols and saponins (Table 1).

Table 1: Presence of phytochemicals in methanolic extract of *Acalypha indica* leaves.

S. No	Phyto constituent	Presence of phyto chemical in methanolic extract
1.	Carbohydrates	+
2.	Flavanoids	+
3.	Alkaloids	+
4.	Tannins	-
5	Phenols	+
5.	Proteins	-
6.	Saponins	+
7.	Sterols	-

+ indicates presence; - indicates absence.

Antibacterial Activity
The methanolic leaf extract of *Acalypha indica* showed substantial inhibitory effect on all of the five bacterial species such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Streptococcus mutans*. The inhibition zones for *S. aureus* were 14 mm, 16 mm and 20 mm (including well 4 mm) at concentrations of 15 µl, 20 µl and 25 µl respectively. The inhibition zones *P. aeruginosa* were 14 mm, 14 mm and 16 mm, 10 mm, 11 mm and 20 mm for *Streptococcus mutans*. The zones for *E. coli* were 10 mm, 12 mm and 21 mm and 14 mm, 16 mm and 19 mm for *Bacillus subtilis*. The anti-bacterial results of methanolic extract were given below (Table 2 and Figure 2-7).

Table 2: Inhibition zones of methanolic extract of *Acalypha indica* against test pathogenic bacteria.

Test bacterial species	Inhibition zones in mm (including well 4mm)		
	15 µl (methanolic extract)	20µl (methanolic extract)	25µl (methanolic extract)
Staphylococcus aureus	14 mm	16 mm	20 mm
Pseudomonas aeruginosa	14 mm	14 mm	16 mm
Bacillus subtilis	14 mm	16 mm	19mm
Escherichia coli	10 mm	12 mm	21 mm
Streptococcus mutans	10 mm	11 mm	20 mm

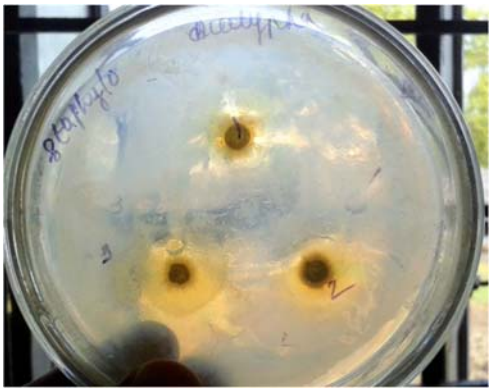


Fig 2: *Staphylococcus aureus*

Fig 3: *E. coli*

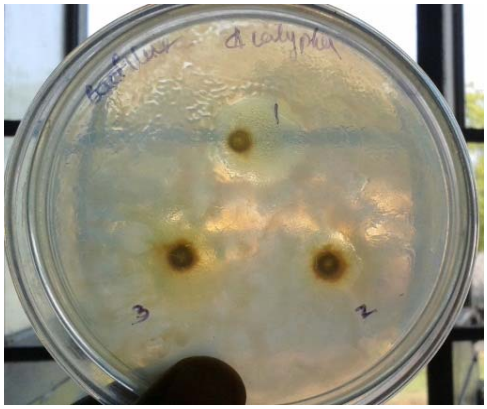
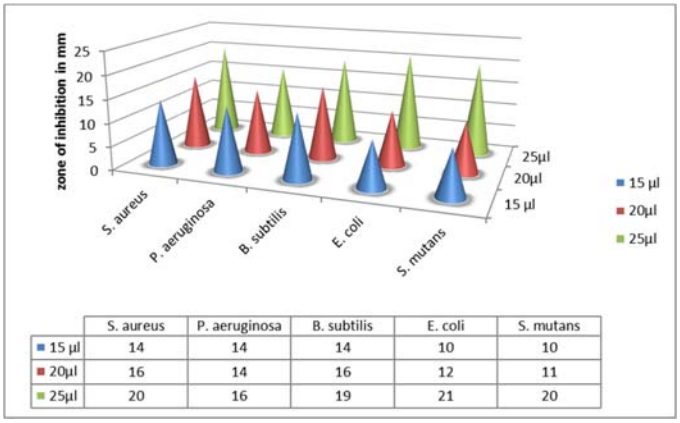


Fig 4: *Bacillus subtilis*

Fig 5: *Pseudomonas aeruginosa*

Fig 6: *Streptococcus mutans*



	S. aureus	P. aeruginosa	B. subtilis	E. coli	S. mutans
15 µl	14	14	14	10	10
20µl	16	14	16	12	11
25µl	20	16	19	21	20

Fig 7: Zones of inhibition of methanolic extract on different bacterial species

Anti hemolytic activity

The methanolic leaf extract of *Acalypha indica* shown to have reasonable anti hemolytic activity. The methanolic extract showed antihemolytic activity in the range from 27% to 54%. At varying concentration of plant extracts 20, 40, 60, 80 and 100 mg /ml the percent of inhibition of hemolysis recorded were 27%, 34%, 40%, 47%, 54% respectively. (Figure 8).

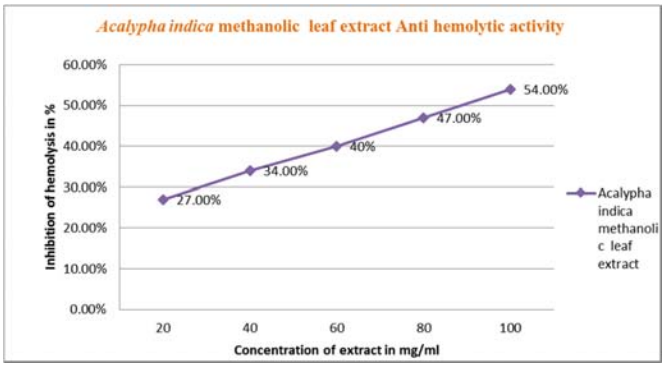


Fig 8: Anti hemolytic activity of methanolic leaf extract of *Acalypha indica*

Discussion

The Methanolic leaf extract of *Acalypha indica* shown to have modest anti hemolytic activity. In this study the inhibition of hemolysis found to be increased with increase in concentration of extract. When red blood cells were treated with H_2O_2 (toxicant), % hemolysis was found to be increased.

This may be because of the oxidizing nature of H2O2 with respect to cell membrane degradation and release of haemoglobin from the cell [17]. H2O2 also cause mobilization of Fe2+ by Ca2+ via Fenton reduction stimulating the production of OH- radicals [18]. All these factors combinedly cause destabilization of cell membrane, which is probably the key event of the lysis of the cell [17]. Polyphenols possess many biological effects, mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelation of transition metals [19, 20]. The methanolic leaf extract of *Acalypha indica* showed inhibitory effect on all of the five bacterial species such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Streptococcus mutans*. The extract has shown inhibitory zones ranging from 10 mm to 21 mm. These findings were in accordance with the recent study by P. Vijayarekha., *et al*., (2015) [1]. In all the bacteria used in this study, *Escherichia coli* proved to be more susceptible and exhibited maximum inhibitory zone 21 mm. The minimum inhibitory zone was observed for *Pseudomonas aeruginosa*. As the modern antibiotics have various anarchic toxic effects, these plant extracts could serve as a alternative antibacterial agents. In single plant many active secondary metabolites are present and medicinal effect can be attributed to either to a single compound or synergistic effect of many compounds and present antibacterial activity can either be due to presence of some specific bioactive molecule or due to the synergistic effect of different phytoconstituents[21].The phytochemical investigation of methanolic extract of *Acalypha indica* leaves showed the presence of carbohydrates, flavanoids, alkaloids, phenols and saponins. Recent phytochemical analysis by Selvamani, Balamurugan., (2015) [22], leaf extracts of *Acalypha indica* recorded the presence of alkaloids, terpenoids, steroids, flavonoids, phenolic compounds, tannins and saponins.

Conclusion

Through this present study it can be concluded that the Traditional healing system has provided many important herbs have the potential to cure lethal diseases. Always the natural drugs are superior than the synthetic drugs in terms of healing as well as efficacy. The feasibility of making efficient drugs from plant sources is very much near. Based on previous studies this plant *Acalypha indica* accounts for various medicinal properties. Hence effective work should be done to isolate of various phyto inhibitors.

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