

Qualitative Phytochemical Screening and Assessment of Antimicrobial Activity of *Acalypha Indica* and its Cytotoxic Studies

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ABSTRACT

The study aimed at qualitative screening of phytochemicals and evaluation of the antimicrobial activity of *Acalypha indica* leaf. Aqueous, chloroform and butanol extracts of dried and ground plant materials were prepared using Soxhlet apparatus. Qualitative phytochemical screening of the extracts showed positive for carbohydrates, chloride, tannins, alkaloids, flavanoids, saponins phenolic compounds and steroids. Antimicrobial activities of the extracts were evaluated by agar well diffusion method. The extracts exhibited significant antimicrobial effects against all the tested bacterial and fungal pathogens. The ethanol extracts of leaf showed highest activity on the Gram positive bacteria *S.aureus* and fungus *A.fumigatus*. The breast cancer (MCF-7) cell lines were used to screen the in vitro anticancer challenging with the *Acalypha indica* extracts. Concentration ranges from 6.25 to 100 µg of *Acalypha indica* purified extracts were screened against (MCF-7) cells. The anticancer activity of *Acalypha indica* were performed by 3-[4, 5-Di methyl thiazol-2-yl]-2, 5-diphenyltetrazolium Bromide (MTT) assay. The cytotoxicity was highly significant in the 100 µg concentration of leaf extract in the breast cancer (MCF-7) cell lines. This study supports the traditional use of *Acalypha indica* for the treatment of various infectious diseases and might be helpful for further investigation of the plants to assess their chemical prospective in future research..

Keywords: Plant extracts, *Acalypha indica*, Phytochemicals, Antimicrobial activity and MTT assay.

1. INTRODUCTION

Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine. The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value [1]. They are the sources of natural pesticides that make excellent leads for new pesticide development [2], [3].

Acalypha indica is an annual herb found throughout various parts of India. This plant has many bioactive potential such as antibacterial, antifungal, antidiabetic, antioxidant and anti inflammatory. The *Acalypha indica* is used for the treatment of diseases such as asthma, cough, dog bite, rheumatism earache scabies scorpion bites, snake bites and sting of centipedes burns and eczema [4]. Pharmacological investigation of *Acalypha indica* has shown that the plant has potent anti bacterial, anti fungal, anti inflammatory, anti osteoporotic, anti-oxidant, neuro protective and wound healing activities [5]. The present study was an attempt made to enrich the knowledge of phytochemical constituents and antimicrobial activity of *Acalypha indica* plant material against human pathogenic bacteria and fungi organisms.

2. MATERIALS AND METHODS

2.1 Plant sample

The plant *Acalypha indica* was collected from Marthandam, Kanyakumari District, Tamil Nadu, India. The leaf of the plant used for this study was rinsed severally with clean tap water and shade dried in a dark place at room

temperature for few days. The dried plant parts were cut into small pieces ground in electric chopper to get fine powder for further use.

2.2 Preparation of extracts

The powdered plant materials were subjected to soxhlet extraction using aqueous, acetone, dimethyl sulfoxide, chloroform and ethanol. Each 5 g of powder of plant material was filled separately in the thimble and extracted successively with 60 ml of solvents using a soxhlet extractor for 3 h. After solvent evaporation, each of these solvent extract was weighed and preserved in room temperature until further use.

2.3 Qualitative analysis of phytochemical constituents

All the extracts were subjected to systematic phytochemical screening for testing the presence of various phytochemical constituents by the method followed by standard protocols [6]. Phytochemical test includes carbohydrates, amino acids, proteins, vitamin C, chloride, tannin, alkaloids, flavonoids, phlobatannins, steroids, phenols and saponins.

2.4 Antimicrobial activity of plant extracts

Antimicrobial activities of the plant extracts were determined by well diffusion method [7]. Four bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Staphylococcus aureus* and three fungal pathogens such as *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium* sp. were used for this investigation. The test bacterial strains were inoculated into Nutrient broth and incubated at 37°C for 24 h. After the incubation period, the culture tubes were compared with the turbidity standard. Fungal inoculums were prepared by suspending the spores of fungus (as previously cultured) in saline water mixed thoroughly, made turbidity standard and used. Fresh bacterial culture of 0.1 ml having 10⁸ CFU was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on Potato dextrose agar. Wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50 µl of plant extracts using micro pipette in aseptic condition. The plates were kept in a refrigerator to allow pre-diffusion of extract for 30 min and then incubated at 37°C for 24 h and 28-30 °C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

2.5 MTT assay

MTT is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilized formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is

a measure of the viability of the cells. The cells was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37°C for 3 hours. MTT was removed by washing with 1x PBS and 200 µl of DMSO was added to the culture. Incubation was done at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank in a micro plate reader (ELISACAN, ERBA).

3. RESULTS

3.1 Qualitative analysis phytochemical constituents

Phytochemical screening of the plant extracts were performed for the analysis of phytochemical constituents In the leaf material, aqueous extract showed positive results for carbohydrate, chloride, tannins, alkaloids, flavonoids, Phlobatannins, steroids, phenolic compounds and saponins; Chloroform extract showed positive results only for chloride, tannins, saponins and phenolic compounds; Butanol extract showed positive results for chloride, tannins and phenolic compounds (Table 1).

Table 1. Qualitative analysis phytochemical constituents

Sl. No.	Chemical constituents	Leaf extracts		
		Aqueous	Chloroform	Butanol
1	Carbohydrates	+	+	-
2	Protein	-	-	-
3	Amino acid	-	-	-
4	Vitamin C	-	-	-
5	Chloride	+	-	+
6	Tannins	+	+	+
7	Alkaloids	+	-	-
8	Flavonoids	+		-
9	Phlobatannins	+	-	-
10	Steroids	+	-	-
11	Phenolic compounds	+	+	+
12	Saponins	+	+	-

‘+’ presence of compound; ‘-’ absence of compound

3.2 Antimicrobial activity of plant extracts

Antimicrobial activity of the plant extracts were determined by well diffusion method against various bacterial and fungal pathogens. The leaf of aqueous extract showed inhibition activity only on *K. pneumonia* (10 mm); chloroform extract showed activity on *E. coli* (10 mm), *S. aureus* (12 mm), *B. cereus* (10 mm), *A. fumigatus* (12 mm) and *A. niger* (13 mm); and butanol extract showed activity on *E. coli* (10 mm), *S. aureus* (14 mm), *B. cereus* (12 mm), *A. fumigatus* (15 mm), *A. niger* (12 mm) and *Penicillium sp* (10 mm) (Table 2).

Table 2. Antimicrobial activity of *Acalypha indica* leaf extracts

Sl. No.	Test organisms	Leaf		
		Aqueous	Chloroform	Butanol
1.	<i>E.coli</i>	-	10mm	10mm
2.	<i>K. pneumoniae</i>	10mm	-	-
3.	<i>S. aureus</i>	-	12mm	14mm
4.	<i>B. cereus</i>	-	10mm	12mm
5.	<i>A. fumigatus</i>	-	12mm	15mm
6.	<i>A. niger</i>	-	13mm	12mm
7.	<i>P. chrysogenum</i>	-	-	10mm

Zone of inhibition in 'mm'

3.3 Anticancer activity

The anticancer effect of *Acalypha indica* leaf extract was determined using MTT assay. The present finding indicated the percentage inhibition of MCF-7 cell lines (Fig.1). The (Fig.2) displayed the anticancer activity of leaf extracts of *Acalypha indica* in MCF-7 cells. The aqueous extract illustrates higher cytotoxicity in 100 µg concentrations. Finally the present study suggests that the aqueous extract of *Acalypha indica* leaf comprised novel anticancer compounds which will be potent therapeutic agent for breast cancers.

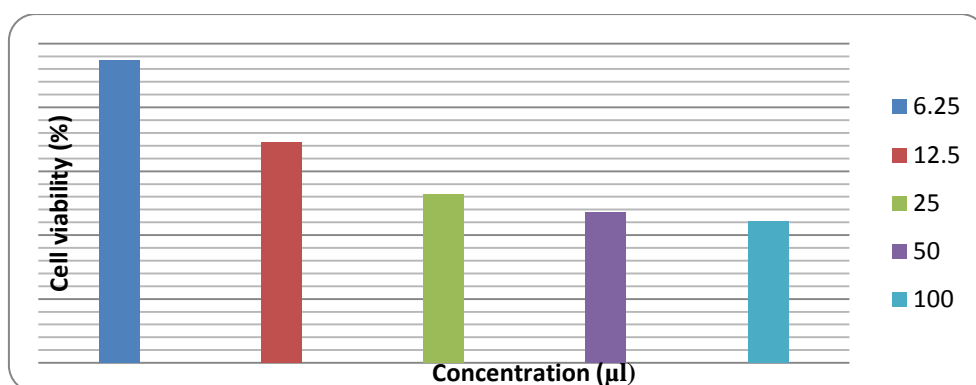


Fig. 1: Percentage viability of MCF 7 cell lines after the exposure with the leaf extract of *Acalypha indica*

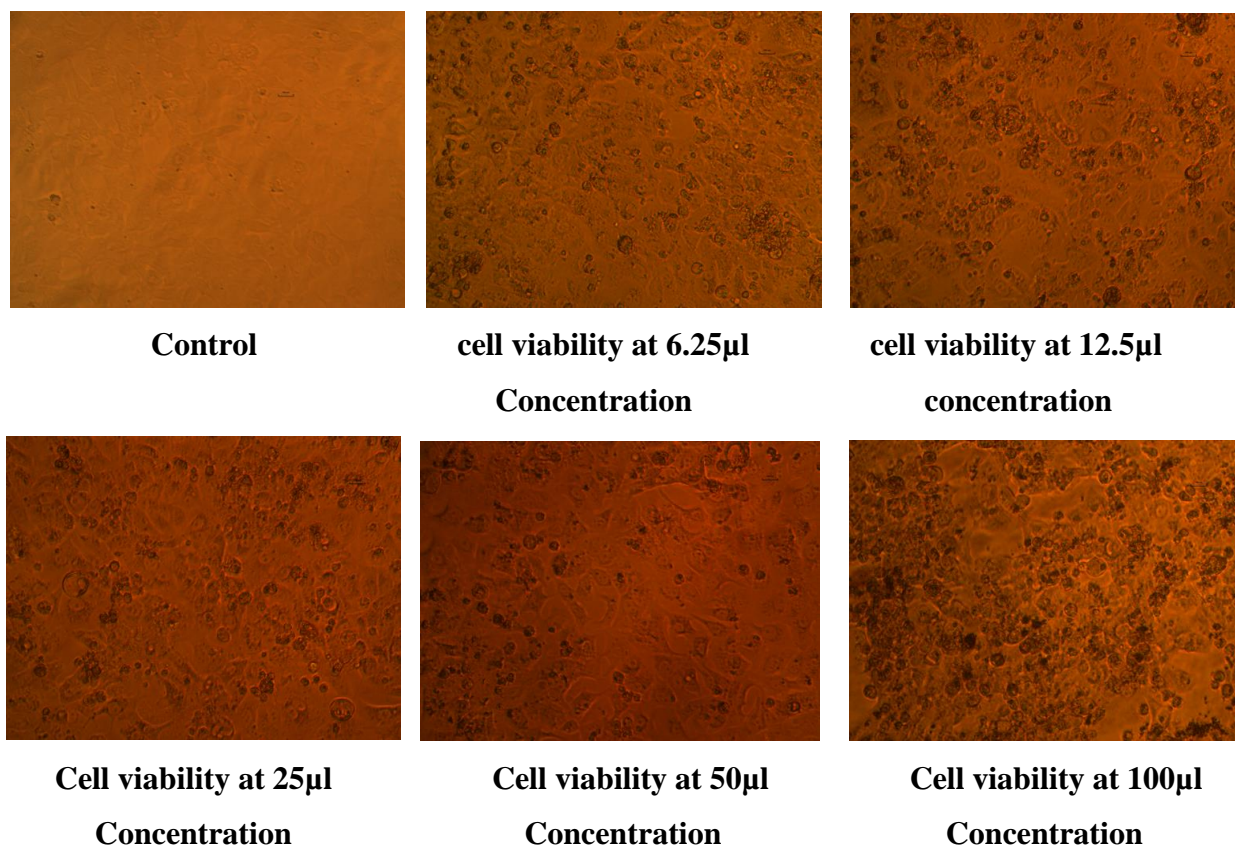


Fig. 2. Anticancer effect of *Acalypha indica* leaf extract on breast cancer cell lines

4. DISCUSSION

The result of the phytochemical screening indicates the presence of carbohydrates, amino acids, chloride, tannins, alkaloids, flavanoids, phenolic compounds and steroids. The pharmaceutical activity of the plant extracts might be due to the presence of secondary metabolites called phytochemicals. The phytochemical constituents of the plant products serve as a defense mechanism [8]. These metabolites possess a broad range of activities, which may help in protection against persistent diseases [9]. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines. For instance, the presence of tannins have astringent properties, which accelerate the healing of wounds and inflamed mucous membrane due to their physiological activities such as anti-oxidant, antimicrobial and anti-inflammatory properties [10]. Flavonoids and resins might be responsible for its use as anti-inflammatory recipe in Chinese folkloric medicine as some flavonoids has anti-inflammatory effect on both acute and chronic inflammation [11].

Phenols are largest group of plant metabolites, which have many biological properties such as antiapoptosis, antiageing, anticarcinogen, anti-inflammation and cell proliferating activities [12]. Plant containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties. Also have a wide range of medicinal applications [13]. In the present study, the plant extracts of *Acalypha indica* inhibited the growth of entire tested pathogenic strains. The butanol extracts of leaf showed highest activity. The antimicrobial of activity

may due to the presence of phytochemical constituents like alkaloids, flavanoids, tannins and steroids. Similarly in the present study *Acalypha indica* were screened for the anticancer activities against breast cancer. The MCF 7 cells were used to study the anticancer activities. A reduction in cell growth and an induction of cell death are two major means to inhibit tumor growth [14]. The result from this work has revealed the medicinal potential of this plant in the treatment of various diseases.

5. CONCLUSION

There are fewer reports available for the study about *Acalypha indica* as an antimicrobial agent. This study supports the traditional use of *Acalypha indica* for the treatment of various infectious diseases in different regions of the world and might be helpful for further investigation of the plants to assess their chemical prospective in future research. Based on the results obtained in this in vitro cytotoxicity study, it can be concluded that the, *Acalypha indica* possesses significant anticancer activity to warrant further extensive study. More work is needed to isolate the bioactive components in the plant.

REFERENCES

- [1] Y. Asakawa, "Biologically active compounds from bryophytes," J Pure Appl Chem, vol. 79, pp. 557-580, 2007.
- [2] S. Satish, K. A. Raveesha, G. R. Janardhana, "Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars," Letters in Applied Microbiology, vol. 28, pp. 145-147, 1999.
- [3] I. Ara, N. A. Bukhari, D. Solaiman, M. A. Bakir, "Antimicrobial effect of local medicinal plant extracts in the Kingdom of Saudi Arabia and search for their metabolites by gas chromatography-mass spectrometric (GC-MS) analysis," Journal of Medicinal Plants Research., vol. 6(45), pp. 5688-5694, 2012.
- [4] Samuel Jacob Inbaneson, Sundaram Ravikumar, Palavesam Suganthi, "In Vitro Antiplasmodial Effect of Ethanolic Extracts of Coastal Medicinal Plants along Palk Strait against *Plasmodium falciparum*," Asian Pacific Journal of Tropical Biomedicine, vol. 2 (5), pp. 364 – 367, 2012.
- [5] Mathew George, Lincy Joseph, Kamal Singh Vyas, Saira Susan Varghese, "Anti Arthritil Activity of Plant *Acalypha indica*," American Journal of pharmacology and pharmacotherapeutics, vol. 3 (3), pp. 007 – 015, 2016.
- [6] J.B Harborne, "Phytochemicals Methods. Chapman and Hall Ltd., London," pp. 49-188, 1973.
- [7] C. Anushia , P. Sampathkumar, L. Ramkumar, "Antibacterial and antioxidant activities in *Cassia auriculata*," Global Journal of Pharmacology, vol. 3(3), pp. 127-130, 2009.
- [8] T. Murugan, J. Albino Wins, M. Murugan, "Antimicrobial activity and phytochemical constituents of leaf extracts of *Cassia auriculata*," Ind J Pharm Sci, vol. 72, pp. 122-125, 2013.

- [9] M. Amin Mir, S. S. Sawhney, M. M. S. Jassal, "Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*," *Wudpecker J Pharma Pharmacol*, vol. 2, pp. 1-5, 2013.
- [10] S. G. Killedar, H. N. More, "Estimation of tannins in different parts of *Memecylon umbellatum* Burm," *J Phar Res*, vol. 3(3), pp. 554-556, 2010.
- [11] O. P Kunle, H. O. Egharevba, "Preliminary studies on *Vernonia ambigua*: Phytochemistry and Antimicrobial Screening of the Whole Plant," *Ethnobotanical Leaflets*, vol. 13, pp. 1216-21, 2009.
- [12] X. Han, T. Shen, H. Lou, "Dietary polyphenols and their biological significance," *Int J Mol Sci*, 8: 950-988, 2007.
- [13] J. Shi, K. Arunachalam, D. Yeung, Y. Kakuda, G. Mittal, Y. Jiang, "Saponins from edible Legumes: Chemistry, processing and health benefits," *J Med Food*, vol. 7, pp. 67-78, 2004.
- [14] Y. T. Huang, D. M. Huang, J. H. Guh, I. L. Chen, C. C. Tzeng, C. M. Teng, "Cil-102 Interacts With Microtubule Polymerization And Causes Mitotic Arrest Following Apoptosis In The Human Prostate Cancer Pc-3 Cell Line," *Journal of Biological Chemistry*, vol. 280, pp. 2771-9, 2005.