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PHARMACOGNOSTICAL EVALUATION, QUALITATIVE ANALYSIS OF PHYTOCHEMICALS AND ANTIMICROBIAL EFFECTS OF DIFFERENT EXTRACTS OF *ACALYPHA FRUTICOSA* LEAVES

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ABSTRACT

Background: *Recently the acceptance of traditional medicine as an alternative source for human health care and the improvement of microbial resistance to the available antibiotics have reaffirmed the need to probe the antimicrobial activity of herbal plants. Acalypha fruticosa is one such plant commonly known as "Chinnichedi" and "Birch leaved acalypha" is a shrub belonging to the family of euphorbiaceae. The Paliyar and Irula tribes in Western Ghats of South India used the leaves and roots of A. fruticosa to treat skin diseases, wounds, stomach ache and poisonous bites.* ***Objectives:*** *Nevertheless, until today, there were no reports to justify its ethnobotanical claim. The objectives of our study were to evaluate the pharmacognostic characters, qualitative phytochemical screening and antimicrobial activity of A. fruticosa.* ***Material and methods:*** *The qualitative phytochemical analysis was performed by standard procedure. The antimicrobial activity of selected medicinal plant was carried out by using disk diffusion method. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) was tested by using tube dilution method.* ***Results & Conclusion:*** *The results of the qualitative phytochemical analysis and fluorescence analysis confirm that this plant is the plentiful source of phytoconstituents. The antimicrobial activity of selected medicinal plants A.fruticosa explicate that it has an effective antimicrobial activity. In bacteria E.coli showed more sensitivity against acetone extract (21 ± 0.3 mm). In fungi the T.simii showed more sensitivity against chloroform extract (22 ± 0.3 mm). Moreover, the acetone extract had more antimicrobial activity than other extracts. In conclusion, we recommend that the plant Acalypha fruticosa apprised here can be used as promising antimicrobial agent in infectious disease treatment.*

KEY WORDS

Antimicrobial activity, phytochemicals, MBC and MFC, pharmacognostic study, Acalypha frutiosa leaves.

1. Introduction

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables, roots and flowers that have defense mechanism and protect from various infections and insects. Phytochemicals are primary and secondary compounds. Chlorophyll, protein and common sugars are included in main constituents and secondary compounds have terpenoid, alkaloid and phenolic compounds (Krishnaiah *et al.,* 2007). The species used for this work, *Acalypha fruticosa* an erect woody shrub, belongs to the family, Euphorbiaceae is one such folklore plant used in traditional system of medicine in throughout the Tamil Nadu, India. This plant is distributed up to 1800m above mean sea level in southern Western Ghats (Gamble and Fischer, 1958). This plant species has been used as a folk medicine for the treatment of dyspepsia, skin complaints, jaundice, cholera, sexually transmitted diseases, stomach problems, antipyretic and even as an antidote (Anandakumar *et al.,* 2009). The stem part of this species is used to cure wounds in animals and also used to treat toothache and the stem is used as fuel wood by tribal people. Despite these uses, no published works are available for this plant. The emergence and spread

of antibiotic resistant microorganisms also triggered this type of plant investigations (Cowan, 1999). Higher plants can serve both as potential antimicrobial crude drugs as well as source of new anti-infective agents (Rios and Reico, 2005). Hence, we undertook the present study, an effort has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. Materials and Methods

2.1 Sample collection

The fresh plant leaves of *Acalypha fruticosa* was collected in the month of November - December in 2015 at the village of Thenkuchipalayam, Villupuram district, Tamilnadu state, India. Then the leaves were washed under running tap water for remove the dust matters and unwanted contaminants. Then the leaves were shade dried and powdered using mechanical grinder. The fine powder was stored in airtight container.

2.2 Extract preparation

The extract was prepared by both hot and cold percolation methods. 20g of dried and powdered plant leaf was soaked in 100 ml of acetone, chloroform and water respectively. Powder with acetone and chloroform solvents were kept at room temperature by occasional shacking for 48hrs (Sharmistha Chakravarthy, Chandra Kalita Jogen, 2012). The water was boiled for over 1 hour at 100^0C. Then the extracts were filtered with Whatmann No.1 filter paper. Then the extract was stored in air tight container then refrigerated at 4^0C for further use.

2.3 Qualitative phytochemical studies

The individual extracts were subjected to different qualitative chemical investigation for the establishing profile of the given extracts for their chemical composition (Raaman, 2006). The crude powder was extracted in different solvents are tested for various phytoconstituents present in them by standard procedure **(**Harborne, 1973; Kokate, 1997). They are generally tested for the presence of alkaloids, flavonoids, tannins, phenols, cardiac glycosides, triterpenes, steroids and saponins.

2.4 Fluorescence analysis test

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and added 1-2 drops of freshly prepared solution, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in various radiations were recorded (Gupta *et al.,* 2006; Kokashi *et al.,* 1958)

2.5 Antimicrobial activity

Antimicrobial activity was determined by disk diffusion method (Kirby *et al.,* 1986).

2.5.1 Preparation of the disc

Each of discs which are approximately 6mm in diameter was cut from Whatman No.1 filter paper. The sterile discs were put in to a three petri dishes and then impregnated with the acetone, chloroform and aqueous extracts by soaking in the extracts for 24 hours. Each of the discs contains acetone, chloroform and aqueous extracts respectively with the recovered from the extract aseptically into the agar surface in a plate.

2.5.2 Determination of antimicrobial activity by disc diffusion method

100 µl of 24 hours old culture of test pathogens were spread on the test plates. Mueller Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. The sterile antibiotic disc in 6 mm diameter impregnated and the Medicament that is respective solvent extracts was loaded in the disc at equidistance. The plates were kept at room temperature for 30 mins, which helps to diffuse the extract on the medium. The test plates were incubated at 37°C for 24 hours to determine the antibacterial activity of the respective solvent extracts of *Acalypha fruticosa* leaf. Ciproflaxocin antibiotic discs were used as positive control for bacteria and Fluconazole were used for fungi. The zone of inhibition (mm in diameter) were read and taken as the activity against the test pathogen.

2.6 Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Sensitivity of bacteria and fungi to different extracts of *Acalypha fruticosa* can be measured by using a tube dilution technique, which determines the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC). These tests were done to determine the lowest concentration of *A.fruticosa* extract, where it can show the bactericidal and

fungicidal effects. Each tube contained an inoculums density of $5x10^5$ CFU/ml of each of the test organisms. All bacteria were grown in Mueller Hinton broth and fungal grown on Potato Dextrose broth. Then the suspension of all the five bacterial culture and three fungal culture was added into tubes containing diluted sample of *A.fruticosa* extract (20 - 100 µg/ml). The dilution of the samples was done with Mueller Hinton broth for bacteria and Potato Dextrose broth for fungi. Finally, the tubes containing diluted sample of *A.fruticosa* and pathogens was then incubated overnight at 37^0C with constant shaking on the shaker. The growth of the microorganisms was determined by turbidity. Clear tubes indicated absence of bacterial growth.

For every experiment, a sterility check, negative control and positive control were included. The MIC of the acetone extract was the lowest concentration in the medium that completely inhibited the visible growth. The solvent value was deducted accordingly to get the final results of activity.

The MBC and MFCs were determined by inoculating the surfaces of Mueller Hinton agar and Saboraud Dextrose agar plates respectively with 25 µl of samples taken from the clear tubes. After the bacterial suspensions had been fully absorbed into the agar, the plates were further incubated at 35^0C for 22hrs and were examined for growth in daylight. The MBC and MFC 50% and 90% were defined as the concentration of drug that resulted 50% and 90% killing of the bacterium and fungi relative to the concentration of pathogens that was present in test wells at 0 hr. After the completion of experimental work, the used microbial strains, media and plastic wares were sterilized and discarded as per the institutional biosafety committee's guidelines.

2.7 Statistical analysis

Results obtained were reported as mean ± SD of triplicate measurements. Significance differences for multiple comparisons were determined by One-way ANOVA followed by Duncan test with P = 0.001 using SPSS (version 19).

3. Results

3.1 Powder characteristics

The dried and powdered leaf was greenish in color and has a distinctive taste in nature. The leaf powder has a very strong odor. When the powder was pressed between two filter papers by mechanically the greasy spot was noted. This indicates the presence of fatty acids. When the powder was mixed with water and shaken well, the development of well froth was noted for one minute. The result indicates the presence of saponin.

3.2 Phytochemical analysis result

The present study has been revealed that the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, hydroxyl anthraquinone, proteins, xantho protein, phlobatannins and emodins were present in the samples. The result of the phytochemical analysis shows that the plant *Acalypha fruticosa* are rich in at least one of the flavonoids, protein, xantho protein, phenols, carbohydrates, cardiac glycosides, fatty acids, phlobatannins and emodins.

3.3 Fluorescence analysis of the powder

The powder was subject to fluorescence analysis as per the standard procedure. The changes in the color of *Acalypha fruticosa* leaf powder under UV radiation in reference to day light were observed with different chemical reagents. This showed different colors of the powder in the presence or absence of chemical constituents. The fluorescence analysis of powdered drug plays a vital role in the determination of quality and purity of drug.

3.4 Fluorescence analysis of the extracts

All three extracts were prepared and then they were treated with reagents and the color changes were observed under day light and UV light (365 nm). All the results are tabulated.

3.5 Results of antimicrobial activity

3.5.1 Antibacterial activity result

The antimicrobial activity of *Acalypha fruticosa* leaf extracts was depicted in (Table 5). All the extracts showed noteworthy activity. Especially *Escherichia coli*, *Streptococcus pneumoniae* and *Micrococcus luteus* showed more sensitive. The inhibition zone length are 21 ± 0.3 mm in *E.coli* on acetone extract, 19 ± 0.8 mm in *K.pneumoniae* in acetone extract 18 ± 0.6 mm in *Micrococcus luteus* on acetone extract and 17 ± 0.8 mm on chloroform extract, 18 ± 0.6 in *Escherichia coli* on chloroform extract. *Streptococcus pneumonia* showed 18 ± 0.3 mm in acetone extract and 17 ± 0.8 mm in

chloroform extract. A minimum inhibition zone was noted in *Klebsiella pneumonia* on aqueous extract.

3.5.2 Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of the *Acalypha fruticosa* leaves extracts

The acetone, chloroform and aqueous extracts *Acalypha fruticosa* leaf were tested against the five bacterial strains. The potent antibacterial effect was noted against *E.coli, K.pneumoniae* and *S.pneumoniae* in acetone extracts. The MIC and MBC values of acetone, chloroform and aqueous extracts was depicted in table 6. The MIC values of acetone extract were in between of 13 µg/ml^{-1} to 18 µg/ml^{-1} whereas the MBC (90%) was in between 38 µg/ml^{-1} to 78 µg/ml^{-1}. The MIC values of chloroform extract were in between of 17 µg/ml^{-1} to 21 µg/ml^{-1} whereas the MBC (90%) was in between 17 µg/ml^{-1} to 48 µg/ml^{-1}. The MIC values of aqueous extract were in between of 26 µg/ml^{-1} to 34 µg/ml^{-1} whereas the MBC (90%) was in between 68 µg/ml^{-1} to 93 µg/ml^{-1}.

3.5.3 Antifungal activity result

In antifungal activity of *Acalypha fruticosa* leaf extracts the *Candida albicans* and *Trichophyton rubrum* showed maximum sensitivity against chloroform extract (Table 7). *T.rubrum* showed a maximum inhibition zone i.e., (22 ± 0.3 mm) and *C.albicans* showed 21 ± 0.3 mm inhibition zone in chloroform extract. A minimum inhibition zone was noted in *T.simii* (7 ± 0.6 mm) in aqueous extract.

3.5.4 Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC) of the *Acalypha fruticosa* leaves extracts

The acetone, chloroform and aqueous extracts *Acalypha fruticosa* leaf were tested against the three fungi. The potent antifungal effect was noted against *C.albicans* and *T.rubrum* in chloroform extracts. The MIC and MFC values of acetone, chloroform and aqueous extracts was depicted in table 8. The MIC values of acetone extract were in between of 18 µg/ml^{-1} to 24 µg/ml^{-1} whereas the MFC (90%) was in between 32 µg/ml^{-1} to 47 µg/ml^{-1}. The MIC values of chloroform extract were in between of 13 µg/ml^{-1} to 21 µg/ml^{-1} whereas the MFC (90%) was in between 27 µg/ml^{-1} to 41 µg/ml^{-1}. The MIC values of aqueous extract were in between of 23 µg/ml^{-1} to 39 µg/ml^{-1} whereas the MFC (90%) was in between 41 µg/ml^{-1} to 78 µg/ml^{-1}.

4. Discussion

Lack of standardization procedures fail to identify the drug from its originality which in that way exploits the handling of drug from its customary system of medicine. Currently, its impact is considerable with treatment failures associated with multidrug resistant bacteria and it has become a global concern to public health (Guschin *et al.,* 2015 and Martin *et al.,* 2015). To determine the antimicrobial activity of *Acalypha fruticosa* leaf crude extracts against the clinical pathogens, zone of inhibition, MIC, MBC and MFC were measured. All the experiments were done in triplicate and results are expressed as mean ± standard error. The plant *Acalypha fruticosa* is used from the ancient time for its great therapeutic values as a remedy in day to day life but in this aspect adulterations are also done which leads to its extinct. In the present study, the qualitative phytochemical screening discovered that the presence of alkaloids, flavonoids, carbohydrates, glycosides, phlobatannins, hydroxyl anthraquinones, proteins, xantho proteins, terpenoids, and emodins in the extracts of *Acalypha fruticosa* leaves. All of these compounds listed above are already well known for their antimicrobial activity. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now (Berdy *et al.,* 2005). Fluorescence is the phenomenon demonstrated by various chemical constituents present in the plant materials. Some components show fluorescence in the discernible range in daylight. The fact that a plant extracts exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to evaluate results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study.

The ultra violet light produces fluorescence in several natural products (e.g. alkaloids like berberine), which do not noticeably fluoresce in daylight. If the substances themselves are not fluorescent, they may often be rehabilitated into fluorescent derivatives or decomposition products by applying diverse reagents. Therefore, some crude drugs are often assessed qualitatively in this technique and it is an imperative parameter of pharmacological evaluation (Gupta *et al.,* 2006; Ansari, 2006). As a result, the process of standardization can be attained by stepwise

pharmacognostic studies as stated above. These studies help in recognition and endorsement of the plant material. Such information can act as allusion information for correct identification of particular plant and also will be useful in conception a monograph of the plant. Further, it will act as a tool to identify adulterants and substituent and will help in maintaining the quality, reproducibility and effectiveness of natural drugs.

The *Acalypha fruticosa* leaf extracts showed quite similar inhibition activity with MIC of 13.26 to 34.78 µg/ml (p-value <0.001) for bacteria and MIC 13.15 to 39.48 µg/ml (p-value <0.001) for fungi depending on the tested microorganisms.

The acetone, chloroform and water extracts of *Acalypha fruticosa* leaf were tested against five different human pathogenic bacteria and three fungi for ensuring antimicrobial activity of *A.fruticosa*. All the extracts were shown considerable activity against the tested pathogens. The results of the antimicrobial study report that all the three extracts of the leaf of *A. fruticosa* generally showed significant activity against the growth of the colonies of five tested bacteria (*Klebsiella pneumoniae, Micrococcus luteus*, *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumoniae*). Among the three extracts, the acetone extract has determined to have highest inhibition activity (21 ± 0.3 mm diameter inhibited zone) against the bacteria (Table 5), *Escherichia coli* followed by the chloroform extract against the bacteria *Klebsiella pneumoniae*, (19 ± 0.8 mm diameter inhibited zone). The minimum inhibition zone was observed on *Staphylococcus aureus* in aqueous extract (10 mm diameter inhibited zone). This is clearly showed that the bacteria *S.aureus* gives some resistance against aqueous extract (Plate 3.9.5). It is explained that the different phytochemicals like cardiac glycosides, carbohydrates, flavonoids, phlobatannins, proteins, emodins and phenolics extracted by using different solvents may be responsible for their antimicrobial effects (Tambekar and Khante, 2010).

The MIC and MBC values of acetone extract of *Acalypha fruticosa* were considerably higher (10 mg ml^{-1}) than the other extracts tested suggesting that these aqueous extracts possess much weaker bactericidal activities (Table 6). In the case of acetone extract of *Acalypha fruticosa* reported an MIC of 13.26 µg/$ml^{-1}$and an MBC (90%) of 38.16 µg/ml^{-1}against *E.coli*, an MIC of 19.33 µg/$ml^{-1}$and an MBC (90%) 44.73 µg/ml^{-1}against *S.aureus*, and an MIC of 16.82 µg/$ml^{-1}$and an MBC (90%) of 86.32 µg/ml^{-1}against *S.pneumoniae,* an MIC of 17.58 µg/ml^{-1} and an MBC (90%) 82.43 µg/ml^{-1} against *M.luteus*, and an MIC 14.23 µg/ml^{-1} an MBC (90%) 31.43 µg/ml^{-1} against *K.pneuminae*. In chloroform extract of *Acalypha fruticosa* reported an MIC of 21.85 µg/ml^{-1} and an MBC (90%) of 47.87 µg/ml^{-1}against *S.aureus*, an MIC of 18.86 µg/$ml^{-1}$and an MBC (90%) 45.32 µg/ml^{-1}against *S.pneumoniae*, and an MIC of 21.36 µg/ml^{-1} and an MBC (90%) of 43.37 µg/ml^{-1} against *E.coli,* an MIC of 18.38 µg/ml^{-1} and an MBC (90%) 48.25 µg/ml^{-1} against *M.luteus*, and an MIC 17.23 µg/ml^{-1} an MBC (90%) 44.29 µg/ml^{-1} against *K.pneumonae*. In aqueous extract of *Acalypha fruticosa* reported an MIC of 26.48 µg/ml^{-1} and an MBC (90%) of 68.32 µg/ml^{-1}against *E.coli*, an MIC of 31.53 µg/$ml^{-1}$and an MBC (90%) 88.32 µg/ml^{-1} against *K.pneumoniae*, and an MIC of 32.24 µg/ml^{-1} and an MBC (90%) of 91.84 µg/ml^{-1} against *E.coli,* an MIC of 32.76 µg/ml^{-1} and an MBC (90%) 92.76 µg/ml^{-1} against *S.aureus*, and an MIC 34.78 µg/ml^{-1} an MBC (90%) 93.37 µg/ml^{-1} against *M.luteus*. The minimum inhibition concentration of the *Acalypha fruticosa* extract was noted in acetone extract against *Escherichia coli* and *Klebsiella pneumoniae.* 13.26 µg/ml^{-1} significantly (p<0.001) inhibits the visible growth of *E.coli* and 14.23 µg/ml^{-1} significantly (p<0.001) inhibits the visible growth of *K.pneumoniae.* 13.26 µg/ml^{-1} of acetone extract of *Acalypha fruticosa* leaf completely (p<0.001) inhibits the visible growth of *E.coli* bacteria. Based on this result, it is clear that the acetone extract of *Acalypha fruticosa* leaf possess significant (p<0.001) antibacterial activity than other extracts.

Plants contain chemical substances that acquire part in the metabolic activities thereby helping to cure the bacterial infections (Despande *et al.,* 2011). Notably acetone and chloroform extracts exposed more activity than the aqueous extract. Furthermore, the acetone extract showed more activity against bacteria and the chloroform extract is given better activity on fungi. The antifungal activity of various extracts of leaf of the species, *A. fruticosa* against the three tested fungal species showed the following results. As seen before in antibacterial activity, the chloroform extract has the highest inhibition activity (22 ± 0.3 mm diameter

inhibited zone) against the fungi, *Trichophyton rubrum* followed by same extract (21 ± 0.3 mm diameter inhibited zone) against the fungi, *Candida albicans,* and acetone extract (18.06 mm diameter inhibited zone) against the fungi, *Candida albicans* (Table 7). Notably all the fungal species showed very high resistance to the standard antifungal agent Fluconazole (Plate 3.9.10).

The MIC and MFC values of chloroform extract of *Acalypha fruticosa* were notably increased than the other extracts. Like antibacterial activity aqueous extract possesses much lower fungicidal activity (Table 8). In acetone extract of *Acalypha fruticosa* reported an MIC of 18.26 µg/ml^{-1} and an MFC (90%) of 32.45 µg/ml^{-1} against *C.albicans*, an MIC of 25.33 µg/ml^{-1} and an MFC (90%) 42.92 µg/ml^{-1} against *T.simii*, and an MIC of 24.88 µg/ml^{-1} and an MFC (90%) of 47.37 µg/ml^{-1} against *T.rubrum*. In chloroform extract of *Acalypha fruticosa* reported an MIC of 13.36 µg/ml^{-1} and an MFC (90%) of 29.42 µg/ml^{-1}against *C.albicans*, an MIC of 13.15 µg/ml^{-1} and an MFC (90%) 29.42 µg/ml^{-1} against *T.simii*, and an MIC of 23.34 µg/ml^{-1} and an MFC (90%) of 41.29 µg/ml^{-1} against *T.rubrum*. In aqueous extract of *Acalypha fruticosa* reported an MIC of 23.34 µg/ml^{-1} and an MFC (90%) of 41.29 µg/ml^{-1}against *C.albicans*, an MIC of 39.48 µg/ml^{-1} and an MFC (90%) 78.65 µg/ml^{-1} against *T.simii*, and an MIC of 39.12 µg/ml^{-1} and an MFC (90%) of 69.78 µg/ml^{-1} against *T.rubrum*. The minimum inhibition concentration of the *Acalypha fruticosa* extract was noted in chloroform extract against *Trychophyton simii* and *Candida albicans*. 13.15 µg/ml^{-1} inhibits the visible growth of *T.simii* 13.36 µg/ml^{-1} inhibits the visible growth of *C.albicans* fungi. The chloroform extract of *Acalypha fruticosa* leaf was showed significant antifungal activity.

This fact indicates the existence of strong antimicrobial activity of leaf part of the study species, *A. fruticosa* and therefore its effective curative property against the infectious diseases caused by bacterial as well as fungal species. The present study on antimicrobial activity reports that the selected species, *A. fruticosa* contains ample variety of active principle compounds to reduce the growth of microbial colonies. It confirms the medicinal value and hence the traditional usage of the study species, *A.fruticosa* against various ailments. Further, these findings may lead support to the traditional use of *A. fruticosa* in the treatment of microbial infections. Further studies are suggested to purify the active compounds for the formulation of new drugs, while go for commercialization.

5. Conclusion

In conclusion, we recommend that the plant *Acalypha fruticosa* apprised here can be used as promising antimicrobial agent in infectious disease treatment. More studies should be extended to this plant to identify more potent extracts in treatment since plant derived substances are potential sources of new antimicrobial agents that may play a more prominent role in integrated disease treatment and remains the only ideal option due to their safety in nature.

Table 1: Loss on drying and ash values of *Acalypha fruticosa* leaf powder

S.No	Parameters	Values of three replicates (% w/w)	Mean ± SEM
		21.6	
1	Loss on drying (LOD)	22.3	21.6 ± 0.062
		21.2	
	Ash values:	16.38	
2	Total ash	16.36	16.86 ± 0.344
		15.20	
		8.43	
3	Acid soluble ash	8.48	8.02 ± 0.332
		7.32	
		6.63	
4	Water soluble ash	6.58	6.32 ± 0.423
		5.87	

Values are expressed as Mean ± Standard Error (P<0.001).

Table 2: Results of qualitative phytochemical analysis of *Acalypha fruticosa* leaf extracts

S.No	Name of the compounds	Name of the solvents		
		Acetone	Chloroform	Water
1	Alkaloids	++	++	--
2	Flavonoids	++	++	++
3	Carbohydrates	+++	++	+
4	Glycosides	+++	+++	+
5	Cardiac glycosides	++	++	+
6	Coumarins	+	+	--
7	Saponin	+	--	+
8	Hydroxy anthraquinones	++	++	+
9	Tannins	--	--	--
10	Phlobatannins	+	--	+
11	Proteins	++	++	+
12	Xantho protein	+++	++	+
13	Amino acids	+	--	--
14	Steroids	--	--	+
15	Terpenoids	+	+	+
16	Phenols	++	-	+
17	Resins	+	+	+
18	Volatile oil	+	--	+
19	Fatty acid	--	--	++
20	Emodins	++	++	+

+ → present in small concentration; ++ → present in moderately high concentration;
+++ → present in very high concentration; -- → absent

Table 3: Results of fluorescence analysis of *Acalypha fruticosa* leaf powder

S.No	Reagents	Day Light	Short UV (254 nm)	Long UV (365 nm)
1	Powder + 1M H_2SO_4	Yellow	Black	Black
2	Powder + 1M HCl	Yellow	Violet	Violet
3	Powder + 10% $CuSO_4$	Green	Violet	Violet
4	Powder + Con.HNO_3	Red	Violet	Violet
5	Powder + Dil.HNO_3	Greenish yellow	Black	Black
6	Powder + Con.HNO_3 + Dil.HNO_3	Reddish brown	Violet	Violet
7	Powder + 10% NaOH	Greenish yellow	Violet	Violet
8	Powder + 1% Glacial acetic acid	Greenish yellow	Violet	Violet
9	Powder + 1% Iodine	Greenish brown	Black	Black
10	Powder + Ethanol	Green	Red	Red

Table 4: Results of fluorescence analysis of *Acalypha fruticosa* leaves extracts

S.No	Chemical Test	Acetone Extract		Chloroform Extract		Aqueous Extract	
		Day Light	UV Light	Day Light	UV Light	Day Light	UV Light
1	Extract + aqu.NaOH 50%	Brown	Purple	Brown	Purple	Orange	Black
2	Extract + alc.NaOH 50%	Dark brown	Purple	Dark reddish brown	Purple	Orange	Black
3	Extract + Con.HCl	Green	Black	Dark green	Purple	Orange	Black
4	Extract + 50% HCl	Greenish black	Dark violet	Green	Purple	Brown	Purple
5	Extract + Con.HNO_3	Greenish black	Dark violet	Red	Black	Orange	Black
6	Extract+ 50% HNO_3	Black	Violet	Greenish brown	Black	Reddish brown	Black
7	Extract + Con.H_2SO_4	Greenish black	Purple	Dark green	Blackish purple	Red	Black
8	Extract+ 50% H_2SO_4	Blackish green	Purple	Brown	Black	Reddish brown	Black
9	Extract+Ammonia solution	Green	Dark violet	Greenish brown	Purple	Orange	Black
10	Extract + 1% Iodine solution	Reddish brown	Black	Brown	Purple	Red	Black
11	Extract + 10% $FeCl_2$	Dark brown	Dark violet	Dark brown	Purple	Reddish black	Black
12	Extract+Glacial acetic acid	Greenish brown	Violet	Reddish brown	Purple	Brownish yellow	Black
13	Extract + Ethanol	Greenish black	Red	Brown	Purple	Brownish yellow	Black

Table 5: Results of antibacterial activity of *Acalypha fruticosa* leaves extracts

S.No	Name of the tested bacteria	Zone of inhibition (mm)		
		Name of the extracts		
		Acetone	Chloroform	Water
1	*Escherichia coli*	21 ± 0.3	18 ± 0.6	16 ± 0.5
2	*Klebsiella pneumonia*	19 ± 0.8	16 ± 0.4	12 ± 0.4
3	*Micrococcus luteus*	18 ± 0.6	17 ± 0.8	13 ± 0.2
4	*Streptococcus pneumonia*	18 ± 0.3	17 ± 0.2	13 ± 0.2
5	*Staphylococcus aureus*	17 ± 0.6	12 ± 0.3	10 ± 0.8

Values are expressed as Mean ± Standard Error ($p<0.001$).

Table 6: MIC and MBC (mean ± SEM) of the *Acalypha fruticosa* leaves extracts against tested pathogens (µg/ml)

Organisms	Concentrations (µg/ml)	Acetone extract	Chloroform extract	Aqueous extract
E.coli	MIC	13.26 ± 0.14	21.36 ± 0.18	26.48 ± 0.43
	MBC (50%)	32.27 ± 0.32	36.27 ± 0.43	49.27 ± 0.38
	MBC (90%)	38.16 ± 0.27	43.37 ± 0.46	68.32 ± 0.64
S.aureus	MIC	19.33 ± 0.45	21.85 ± 0.19	32.76 ± 0.23
	MBC (50%)	37.56 ± 0.78	38.87 ± 0.42	79.38 ± 0.61
	MBC (90%)	44.73 ± 0.86	47.87 ± 0.12	92.36 ± 0.61
K.pneumoniae	MIC	14.23 ± 0.76	17.23 ± 0.68	31.53 ± 0.28
	MBC (50%)	27.87 ± 0.11	35.83 ± 0.12	76.83 ± 0.13
	MBC (90%)	31.43 ± 0.54	44.29 ± 0.70	88.32 ± 0.48
S.pneumoniae	MIC	16.82 ± 0.16	18.86 ± 0.10	32.24 ± 0.67
	MBC (50%)	78.70 ± 0.22	38.24 ± 0.75	78.43 ± 0.35
	MBC (90%)	86.32 ± 0.42	45.32 ± 0.47	91.84 ± 0.12
M.luteus	MIC	17.58 ± 0.41	18.38 ± 0.53	34.78 ± 0.14
	MBC (50%)	73.69 ± 0.22	34.54 ± 0.32	79.87 ± 0.11
	MBC (90%)	82.43 ± 38	48.25 ± 0.72	93.37 ± 0.42

Values are expressed as Mean ± Standard Error ($p<0.001$).

Table 7: Results of antifungal activity of *Acalypha fruticosa* leaves

S.No	Name of the fungi	Zone of inhibition (mm)		
		Name of the extracts		
		Acetone	Chloroform	Water
1	*Candida albicans*	18 ± 0.6	21 ± 0.3	12 ± 0.7
2	*Trichophyton rubrum*	14 ± 0.3	22 ± 0.3	11 ± 0.5
3	*Trichophyton simii*	13 ± 0.3	12 ± 0.8	7 ± 0.6

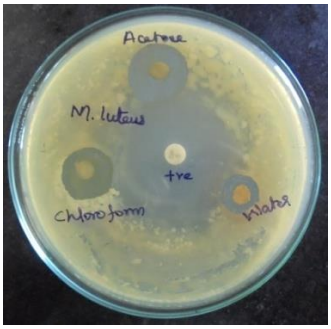
Values are expressed as Mean ± Standard Error ($p<0.001$).

Table 8: MIC and MFC (mean ± SEM) of the *Acalypha fruticosa* leaves extracts against tested pathogens (µg/ml)

Strains	Concentrations (µg/ml)	Acetone extract	Chloroform extract	Aqueous extract
	MIC	18.26 ± 71	13.36 ± 0.38	23.34 ± 0.48
C.albicans	MFC (50%)	27.18 ± 0.71	21.78 ± 0.10	37.72 ± 0.17
	MFC (90%)	32.45 ± 0.38	29.42 ± 0.39	41.29 ± 0.74
	MIC	25.33 ± 0.48	13.15 ± 0.82	39.48 ± 0.41
T.simii	MFC (50%)	33.47 ± 0.39	19.63 ± 0.22	53.74 ± 0.13
	MFC (90%)	42.92 ± 0.03	27.34 ± 0.67	78.65 ± 0.22
	MIC	24.88 ± 0.10	21.32 ± 0.69	39.12 ± 0.84
T.rubrum	MFC (50%)	32.64 ± 0.31	32.16 ± 0.88	52.35 ± 0.54
	MFC (90%)	47.37 ± 0.61	41.73 ± 0.20	69.78 ± 0.13

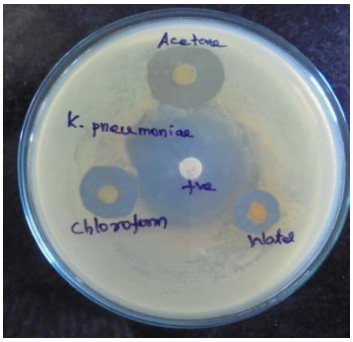
Values are expressed as Mean ± Standard Error (p<0.001).

Fig.1 Plates of antibacterial activity of *Acalypha fruticosa* leaves extracts

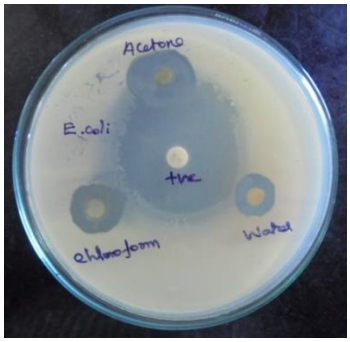


Micrococcus luteus

Staphylococcus aureus



Klebsiella pneumoniae

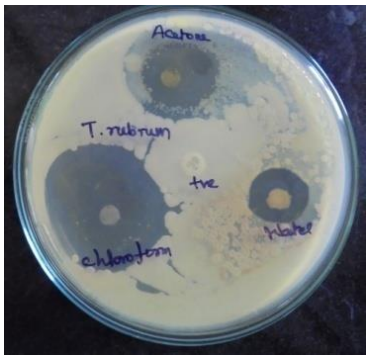


Escherichia coli

Streptococcus pneumoniae

Fig.2 Plates of antifungal activity of *Acalypha fruticosa*

Candida albicans *Trichophyton simii*



Trichophyton rubrum

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