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ANTIMICROBIAL PROPERTIES OF *ACALYPHA INDICA* LINN FLOWERS: AN IN VITRO INVESTIGATION

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ABSTRACT
The well diffusion method was used to inhibit the bacterial and fungal growth. Among the solvent extracts tested, ethanol extract inhibited the growth of all the tested bacteria and fungi having various degrees ofinhibition zones. The aim of present study is to find out the antimicrobial activity and Minimum Inhibitory Concentration (MIC) of Acalypha indica against bacterial isolates (Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Streptomyces griseus)and fungal isolates (Aspergillus niger, Penicillium chrysogenum, Candida albicans and Trichoderma reesei). Powered flowers was prepared and used for extraction with various solvents, viz, the ethanol, hydro-ethanol and chloroform extract of the A.indica. All the solvent extracts were evaporated to dryness. Highest bacterial inhibitory activity was observed with ethanol extract of flower against S. aureus (34 mm), followed by B. subtilis (27 mm)and minimum inhibitory concentration was observed 0.11mg/ml also against S. aureus. On the other hand, maximum inhibition zone against fungi was determined with
P. chrysogenum (22 mm) and 0.33 mg/ml strong MIC was observed against P. chrysogenum by ethanol extract of flower. Both results were observed in case of ethanol extract. The study demonstrated that the ethanol extract of Acalyphaindica flower is potentially good source of antibacterial agents as compared to other extracts. Hence further in vivo evaluation is necessary to identify the specific bioactive compounds, their mode of action and their nontoxic nature.

Keywords: Minimum Inhibitory Concentration (MIC), Acalypha indica, Antimicrobial activity

INTRODUCTION
Various medicinal plants have been used for years in daily life to treat diseases all over the world. Plants produce a diverse range of bioactive molecules. Higher plants as source of medicinal compounds to play a dominant rolein the maintenance of human health since ancient times (Singhet al., 2011). Infectious diseases represent a serious health problem and account for one third of all deaths worldwide (Perumalet al., 2012). The use of plant materials for medicines has a long history, since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings as well as livestock (Kalayouet al., 2012). Nature is a source of medicinal agents and these agents have been used for thousands of years and number of modern drugs has been isolated from natural sources (Kiranet al., 2011). Along with microorganisms are developing their resistance to many commercial antibiotics and that is the major cause of failure to treat various infectious diseases. Therefore,

immense clinical problem in the treatment of infectious diseases has been raised (Islamet al., 2010).Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants could be an excellent source of drugs to fight off this problem (Manandharet al., 2019). Antimicrobials of plant have enormous therapeutic potential as they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Tomokoet al., 2002).Natural antimicrobial agents can act alone or in combination with antibiotics to enhance antimicrobial activity against a wide range of microbes (Bazzazet al., 2016; Bazzazet al., 2018). Plants are rich in a wide variety of secondary metabolites, such as terpenoids, tannins, alkaloids, flavonoids, saponins and anthraquinones which have antimicrobial properties (PriyaDarsini, 2015).In traditionalmedicine, there are many natural crude drugs that have the potential to treat many disease and disorders one of them is Acalyphagenuses that show a great potential in the world of scientific advancement due to its promisingchemical and biological results (Malpaniet al., 2012; Ishaket al., 2013).The previous review of Nag et al. in 2018, they have highlighted the review articles of antimicrobial activity of Acalyphaindica L. in which they reported that this plant contains various bioactive complexes as like the acalyphamide, amide along with a few further 2-methylanthraquinone, amides, y-sitosterol, β sitosterol, tri-O-methyl ellagic acid and stigmasterol withtheir antimicrobial roles.

The various parts of the plant (leaves, roots, seeds, seed oil, stalk and flowers) are widely used in a variety of ailments in traditional system of medicine such as Ayurveda and Siddha.The plants are emetic, expectorant, laxative and diuretic. It is useful in bronchitis, pneumonia, asthma and pulmonary tuberculosis (Chaudhariet al., 2017). Acalyphaindica Linn. (Euphorbiaceae) also known as ‘kucinggalak’ is widely distributed throughout tropical Africa and South Africa, India and Sri Lanka, as well as Yemen and Pakistan. It is a monoecious plant with a weedy nature, annual to sometimes short-lived perennial herb that can grow up to 1.5 to 2.5 m tall (Ishaket al., 2013). A. indica are popularly utilized as herbal medicine in the Indian Ocean islands as well as in India for its expectorant properties. Acalyphaindica is an erect, often simple-stemmed herb and has been acknowledged by local people as a useful source of medicine for several therapeutic treatments (Naveenaaet al., 2020). It hasanticancer, antidiabetic, hepatoprotective, and anti-ulcers activity (Chekuriet al., 2020).A. indica was also shown to possess anti-microbial and anti-inflammatory activities (Rahman et al., 2010; Mickymaray 2019) and was described as an antioxidant agent (Priya and Bhaskara Rao, 2016).Its leaves used for the prevention and reversal of atherosclerotic disease. Powdered dried leaves used for bed sores. Juice of, fresh leaves, mixed with oil or lime are used for rheumatic complaints(PriyaDarsini, 2015).A. indica has also been used traditionally for the treatment of throat infections, wound healing, anti-venom and migraine pain relief. There are various clinical constituents namely kaempferol glycoside, mauritianin, clitorin, nicotiflorin and biorodin that have been isolated from the flower and leaves of A. indica (Nahrstedtet al., 2006). The medicinal properties and therapeutic uses of Acalyphaindica and its secondary metabolites investigations prove its importance as a valuable medicinal plant (Chekuriet al., 2020).Hence, the present study was carried out to analyze antimicrobial activity and Minimum Inhibitory Concentration (MIC) of ethanol, hydro-ethanol and chloroform extract of the A.indicaflowers.

MATERIAL AND METHOD

1) COLLECTION OF PLANT MATERIALS

The flowers of Acalypha indica as experiment plant material were procured from Botanical Garden of the Glocal University, Uttar Pradesh India.

2) PREPARATION OF PLANT EXTRACTS

The freshly collected flower part of A.indica have been washed thoroughly with tap water and are subjected to air-drying in the shade at room temperature (32-37°C) for about 2-3 weeks. The dried plant sample was

ground into powder form by using a homogenizer. About 50gm of powdered plant material (50gm/250mL) was extracted in a Soxhlet extractor for 8 to 10 hours, sequentially with ethanol, hydro-ethanol, and chloroform. Theextracts obtained were then concentrated and finally dried to a constant weight. Dried extracts were kept at20°C until further tests were carried out.

3) ANTIMICROBIAL ACTIVITY

Microbial Strains, culture medium and inoculum preparation:

Clinical laboratory bacterial isolates of Staphylococcus aureus MTCC 3381, Streptomyces griseus MTCC 4734, Bacillus subtilisMTCC10619and Escherichia coli MTCC 443, and fungal isolates viz. Candida albicans MTCC 183, Aspergillus niger MTCC 872, Trichoderma reesei MTCC 164and Penicillium chrysogenum MTCC 5108 were collected from the stock cultures of IMTECH Laboratory, Chandhigarh, India.

Determination of Antibacterial Assay

Mueller Hinton agar no. 2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations of 10 mg/mL. In vitro antibacterial activity of the crude extracts were studied against gram positive and gram negative bacterial strains by the agar well diffusion method (Perez et al., 1990). The Mueller Hinton agar was melted and cooled and then poured into sterile petri dishes to give a solid plate. A standardized inoculum (1.5×108 CFU/mL, 0.5 McFarland) prepared in sterilized 0.9% saline water was used. Wells were prepared in the seeded agar plates. The test compound (60 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37ºC. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The experiment was performed three times to minimize the error and the mean values are presented. The diameters of zone of inhibition produced by the agent were compared with those produced bythe commercial control antibiotics, Ciprofloxacin (40 µl). The control zones were subtracted from the test zonesand the resulting zone diameter was measured with antibiotic zone reader to nearest mm.

Determination of Antifungal Assay

Anti-fungal activity of the experimental plant was investigated by agar well diffusion method (Bonjaret al., 2005). The fungi were subculture onto Sabouraud's dextrose agar, SDA (Merck, Germany) and respectively incubated at 37°C for 24 h and 25°C for 2-5 days. Suspensions of fungal spores were prepared in sterile PBS and adjusted to a concentration of 106 cells/ml. Dipping a sterile swab into the fungal suspension and rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter were punctured in the culture media using sterile glass tube. 60 µlof fresh extracts was administered tofullness for each well. Plates were incubated at 37°C. After incubation of 24 h bioactivities were determined by measuring the diameter of inhibition zone (in mm). Ketoconazole (40 µl) was used as antifungal positive control.The All experiments were made in triplicate and means were calculated.

Minimum Inhibitory Concentration (MIC)

The MIC determination of the crude extracts was referred and modified from (Lalitha, 2005; Wiegandet al., 2008). 60 µL samples were diluted by two-fold serial dilution with its suitable solvent in the wells of a microtiter plate. 240 µL of the prepared microbial culture was added to each well to give a final volume of

300 µL with final concentrations of each well ranging from 0.96 mg/mL to 0.11 mg/mL. The microtiter plates were then incubated at 30°C to 37°C for 24 to 48 hours, with their upper surface covered and sealed with parafilm. After incubation, 20 µL of MTT solution was pipette into the wells for indication of growth. The lowest concentration that shows any visible growth was recorded as the MIC of that extract for the tested microbial species. All the MIC experimentations were performed in triplicate.

RESULTS AND DISCUSSION

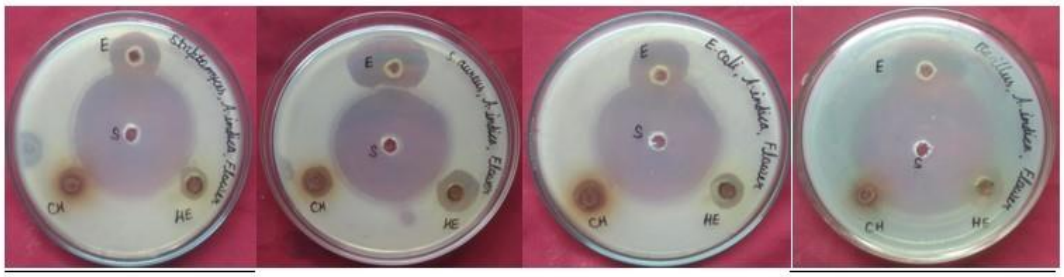
Antibacterial Activity

Bacterial infections contribute heavily to serious complications and can lead to early death in patients (Ganesan and Xu, 2018).Medicinal plant-derived compounds could provide novel straightforward approaches against pathogenic bacteria (Vaouet al., 2021).Specific medicinal compounds in Acalypha indica are responsible for bacterial inhibition. Batubaraet al., 2016 reported that the flavonoids, tannins, polyphenol, protein and saponinplay an important role in inhibiting and retarding bacterial growth. In the present study, the results for the antibacterial activity test of different crude solvent extracts of A. indica parts were displayed in Table 1-4. Inantibacterial activity, all ethanol, hydro-ethanol and chloroform extract of A. indica flower showed maximum zone of inhibition against Staphylococcus aureus, Streptomyces griseus, Bacillus subtilis and Escherichia coli bacteria. As compared to other extracts, flower ethanol extract gave significant results against all studied bacteria. The ethanol extract of flower exhibited highest (34 mm) antibacterial zone against S. aureus, followed by 27 mm, 23 mm and 21 mm against B. subtilis, E.coli and S. griseus. The hydro-ethanol extracts was showed 18 mm, 16 mm, 14 mm and 13 mm inhibition diameter against S. aureus, B. subtilis, E.coli and S. griseus. Whilechloroform extract showed lowest inhibition activity against all tested bacteria. All microbes were susceptible toits respective positive control. Chekuriet al., 2016 and Suresh et al., 2016 reported antibacterial activity of different fractions from A. indica against S. aureus, P. aeruginosa, E. coli and Bacillus species. Ethanol extract of Acalyphaindica showed more potency against Staphylococcus aureus with an inhibition zone of 12.46 mm (Chekuriet al., 2018).Similarly the ethanolic extract of A.indicashowed very good zones of inhibition for the microorganisms (Shaliniet al., 2018). Singh, 2019 also observed its significant antimicrobial properties by disc diffusion assay and micro-broth dilution assay against tested pathogen such as Staphylococcus epidermidis, Staphylococcus epidermidis, Streptococcus pyrogens, Salmonella typhi, Bacillus cereus, Escherichia coli, P. aeurigonosa, S. aureus. Madhavan, 2021revealed 7 major bioactive compounds in phytochemical screening of A. indica leaf by GC-MS and a large portion of the restorative properties were 1H-Pyrrole-2,5-dione,1-ethenyl-,3,8-Nanodiene-2-one,(E)-, Proline,3,4-dehydro-, 4-Amino-3-methoxypyrazolo [3,4- d]pyrimidine,Propanenitrile,3-(5-diethylamino-1-methoxy-3-pentynyloxy)-mixes among 7 bioactive compounds. He concluded that extracts of Acalyphaindicamay be useful in formulating and synthesizing new antibacterial drugs. Chekuriet al., 2020 reported that it a rich source of glycosides, flavonoids and tannins which shows therapeutic potential. According to a study conducted by Govinda rajanet al., 2008, A. indica extracts produced active results against all the Gram-positive bacteria tested. This result could be attributed to the difference in wall compositions that exist in both Gram-positive and Gram-negative bacteria. While the Gram- negative bacteria possess wall that consists of lipopolysaccharide layer along with proteins and phospholipids that may impede the entry of active compounds of A. indica crude

extracts, the Gram-positive bacteria contains a very active area of cell metabolism called periplasmic space that carry many digestive enzymes and transport proteins which could attribute to the susceptibility of the microorganisms. Hence, maximum zone of inhibition was observed with gram-positive bacteria.

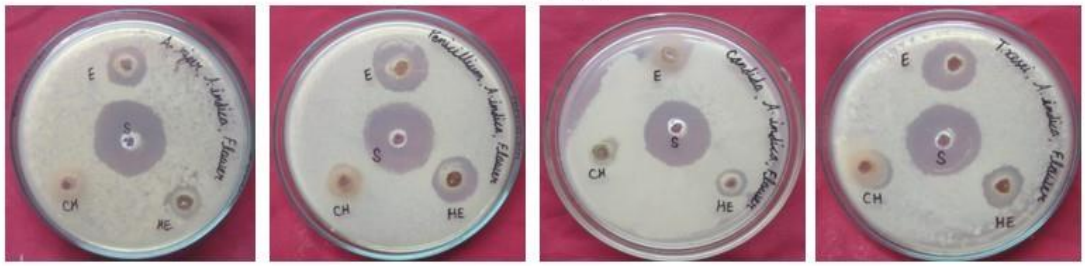
Antifungal activity

As similar to antibacterial results, highest anti-fungal activity was observed with ethanol extract of flower against C.albicans, A. niger, T.reeseiand P. chrysogenum as shown in Table 5-8.The ethanol extract of flower was showed maximum 22 mm diameter of zone of inhibition against P. chrysogenum, followed by 20 mm inhibition zone against T. reesei. The minimum inhibition zone (12 mm) was observed with chloroform extract against A. niger and C. albicans, respectively. Thus, in the aspect of antifungal properties of A. indica, the plants extract prove to be more active against fungi P. chrysogenum and T.reeseiin comparison with C. albicans and A. niger. Previous antifungal studies conducted by Sureshet al., 2009; Kanimozhiet al., 2012) proved that methanol extract of A. indica possess antifungal activity against C. albicans. However, their studies involve the use of the whole plant and not a specific part of the plant. Another study from Solomon et al., 2005 has also showed that the methanol leaves extract of A. indica is most active against C. albicans compared to other extracts tested as it resulted in the highest inhibition zones against fungi. Singh, 2019 observed significant antimicrobial properties of this plant by disc diffusion assay and micro-broth dilution assay against tested pathogen such as A. fumigatus, A. flavus, A. niger, C. albicans and C. tropicals. Ethanol extract of A. indica showed prominent antifungal activity against candida albicans with an inhibition diameter of 12.53 (mm) and Aspergillus niger with a diameter of 9.21 (mm) when compared to other methanol, acetone and chloroform extracts (Chekuriet al., 2018).Hnatyszynet al., 2007 expected that the phenols and flavonoids in A.indicaare a source of anti-fungal activity based on the previous studies. For anti-fungal activity, extraction from water like adecoction is recommended since fungi are affected by any photochemical drugs (Chekuriet al., 2020). Raw of the whole plant is suggested to treat fungal infections in the human body. In a study conducted by Somchitet al., 2010, C. albicans was also proven to be susceptible towards chloroform and ethanol extracts compared to other fungi. Thus, findings from current study support the use of Acalypha indica in traditional medicine for the treatment of various bacterial and fungal infections.



(A) (B) (C) (D)

Figure 1: Antibacterial activity of floweragainst (A)Streptomycesgriseus(B)Staphylococcus aureus(C) Escherichia coliand (D)Bacillus subtilis



(A) (B) (C) (D)

Figure 5: Antifungal activity offlower againstAspergillus niger, Penicilliumchrysogenum, Candida albicans, Trichoderma reesei

Table 1: Antibacterial activity of flower against Bacillus subtilis

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	13
Ethanol	22	27
Hydro-ethanol	22	16

Table 2: Antibacterial activity of flower against Escherichia coli

Extracts	Inhibition zone (mm)	
	Positive	Flower
	Control	
Chloroform	22	13
Ethanol	22	23
Hydro-ethanol	22	14

Table 3: Antibacterial activity of flower against Staphylococcus aureus

Extracts	Inhibition zone (mm)

	Positive Control	Flower
Chloroform	22	14
Ethanol	22	34
Hydro-ethanol	22	18

Table 4: Antibacterial activity of flower against Streptomyces griseus

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	12
Ethanol	22	21
Hydro-ethanol	22	13

Table 5: Antifungal activity of flower against Aspergillus niger

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	12
Ethanol	22	16
Hydro-ethanol	22	13

Table 6: Antifungal activity of flower against Penicilliumchrysogenum

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	14
Ethanol	22	22
Hydro-ethanol	22	19

Table 7: Antifungal activity of flower against Candida albicans

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	12
Ethanol	22	15
Hydro-ethanol	22	13

Table 8: Antifungal activity of flower against Trichoderma reesei

Extracts	Inhibition zone (mm)	
	Positive Control	Flower
Chloroform	22	15
Ethanol	22	20
Hydro-ethanol	22	18

Minimum Inhibitory Concentration

As it is showed in Table 9-15, ethanol extract followed by hydro-ethanol extract recorded a strong MIC value of 0.11 mg/mL and 0.13 mg/mL, respectively with flower in which the growth of bacterial isolate S. aureus was inhibited while the chloroform extract was recorded to possess a medium MIC value of 0.26 mg/against S. griseus and 0.43 mg/mL against A. niger and C. albicans fungi. Singh, 2019 found chloroform extract as a least active against the fungal pathogens as compared to aqueous, methanol and acetone fractions. The ethanol fractions of A. indica exhibited antibacterial activity against E. coli and K. pneumoniae and Carbapenem resistant A. baumannii, K. pneumonia and P. aeruginosa with MIC between 512 to 1024µg/mL (Sureshet al., 2021).The antimicrobial activity could be attributed to the presence of alkaloids, tannins and saponins in the A. indica leaf according to (Rajaselvamet al., 2012). This was also supported by (Khaleel andSudarshanam, 2011) with additional compounds that were discovered such as steroids, cardiac glycosides and phenols. According to a study conducted by Mohan et al., 2012 the presence of bioactive compounds such as alkaloids, tannins, steroids, saponins, flavanoids, glycosides and phenolic compounds was also detected during it phytochemical testing.

Table 9: Minimum inhibitory concentration of flower against Bacillus subtilis

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.23
Ethanol	0.14
Hydro-ethanol	0.18

Table 10:Minimum inhibitory concentration of flower against Escherichia coli

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.24
Ethanol	0.15
Hydro-ethanol	0.17

Table 11:Minimum inhibitory concentration of flower against Staphlococcus aureus

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.21
Ethanol	0.11
Hydro-ethanol	0.13

Table 12:Minimum inhibitory concentration of flower against Streptomyces griseus

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.26
Ethanol	0.19
Hydro-ethanol	0.23

Table 13:Minimum inhibitory concentration of flower against Aspergillus niger

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.43
Ethanol	0.35
Hydro-ethanol	0.37

Table 14:Minimum inhibitory concentration of flower against Penicilliumchrysogenum

Extracts	MIC (mg/mL)
	Flower
Chloroform	0.41
Ethanol	0.33
Hydro-ethanol	0.34

Table 15: Minimum inhibitory concentration of flower against Candida albicans

Extracts	MIC (mg/mL)
	Flower

Chloroform	0.43
Ethanol	0.36
Hydro-ethanol	0.39

Table 16: Minimum inhibitory concentration of flower against Trichoderma reesei

Extracts	**MIC (mg/mL)**

	Flower
Chloroform	0.42
Ethanol	0.34
Hydro-ethanol	0.33

CONCLUSION

In conclusion, the present study indicates that the all extract of A. indica flowers possess some antimicrobial activities against certain pathogenic microbes. The extracts of ethanol, followed by hydro-ethanol flower proved to be a good antibacterial and antifungal agent against Staphylococcus aureus and Penicillium chrysogenum as compared to chloroform extracts. Nevertheless, future studies in regards to its bioactive compound should be done in order to identify the compound that is responsible for its antimicrobial activities. In addition, invivo antimicrobial activity is also necessary to prepare herbal formulation of Acalyphaindica for its fight against infectious microbes.

REFERENCES

1. Batubara, I., Wahyuni, W.T., Firdaus, I. (2016). Utilization of Anting-Anting (Acalyphaindica) leaves as antibacteria. IOP Conference Series: Earth and Environmental Science. IOP Publishing.2016; 012038.
2. Bazzaz, B.F.,Sarabandi, S., Khameneh, B.,Hosseinzadeh, H. (2016). Effect of Catechins, Green Tea Extract and Methylxanthines in Combination with Gentamicin against Staphylococcus aureus and Pseudomonas aeruginosa—Combination Therapy against resistant bacteria. J. Pharmacopunct., 19: 312–318.
3. Bazzaz, B.F., Khameneh, B., ZahedianOstad, M.R.,Hosseinzadeh, H. (2018). In vitro evaluation of antibacterialactivity of verbascoside, lemon verbena extract and caffeine in combination with gentamicin against drug- resistant Staphylococcus aureus and Escherichia coli clinical isolates. Avicenna J. Phytomed., 8: 246–253.
4. Bonjar, S., Aghighi, S., Karimi, N.A. (2005). Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of South East Regions of Iran. J. Biol. Sci.,4: 405-412.
5. Chaudhari, Y.K., Jagtap, V.A., Kanase, V.G., Ansari, I. (2017). To Study The In Vitro Anti-Inflammatory Activity of AcalyphaIndicaLeaves.World Journal of Pharmaceutical Research, 6(2): 650-660.
6. Chekuri, S., Vankudothu, N., Panjala, S., Babu Rao, N., Anupalli, R.R. (2016). Phytochemical analysis, anti- oxidant and anti-microbial activity of Acalyphaindica leaf extracts in different organic solvents. Int.

J. Phytomedicine, 8: 444.

7. Chekuri, S.,Jyoti, B.A., Sompaga, S.,Panjala, S., Anupalli, R.R.(2018). Evaluation of anti-microbial and anti- fungal activity of Acalyphaindica L. Leaf Extract. International Journal of Pharmacognosy and Phytochemical Research, 10(1): 48-51.
8. Chekuri, S., Lingfa, L., Panjala, S., Bindu, K.C.S., Anupall, R.R. (2020). Acalyphaindica L. – an Important Medicinal Plant: A Brief Review of Its Pharmacological Properties and Restorative Potential. European Journal of Medicinal Plants, 31(11): 1-10
9. Ganesan, K. and Xu, B. (2018). Molecular targets of vitexin and isovitexin in cancer therapy: a critical review. Ann. N. Y. Acad. Sci., 1401: 102–113.
10. Govindarajan, M.,Jebanesan, A.,Reetha, D.,Amsath, R., Pushpanathan, T.,Samidurai, K. (2008).Antibacterial activity of Acalyphaindica L. Eur. Rev. Med. Pharmacol. Sci., 12: 299-302.
11. Hnatyszyn, O.,Juárez, S., Ouviña, A., Martino, V., Zacchino, S., Ferraro, G. (2007).Phytochemical analysis and antifungal evaluation ofSebastianiacommersonianaextracts.Pharm. Biol., 45:404-406.
12. Ishak, F.D., So'ad, S.Z.M., Jauhari, A.H.A., Mashud, N.N., Hassan, N.M. (2013). In Vitro Study of Antimicrobial Activity of AcalyphaIndica Linn. Extract. The Open Conference Proceedings Journal, 4(Suppl-2,M14): 57-60.
13. Islam, S., Rahman, A., Sheikh, M.I., Rahman, M., Jamal, A.H.M., Alan, F. (2010).In vitro Antibacterial Activity of Methanol Seed Extract of Elettariacardamomum (L.)Maton.Agriculturae Conspectus Scientificus., 75(3): 113-117.
14. Kalayou, S., Haileselassie, M., Gebre-egziabher, G., Tiku, T., Sahle, S., et al.(2012).In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. Asian Pacific Journal of Tropical Biomedicine, 512-522.
15. Kanimozhi, D., Rathabai, V. and Baskaran, C. (2012).Evaluation of anti microbial activity of Acalyphaindica. Int. J. Res. Pharm. Sci., 2(1): 129-137.
16. Khaleel, B.S. andSudarshanam, G. (2011).Multiple herbal therapy - antimicrobial activity of wound healing paste (Pasuru) used by Sugali tribes of Yerramalais of Kurnool district,Andhra Pradesh,India. Int. J. Pharm. Technol. Res., 3(3): 1238-1241.
17. Kiran, B., Baruah, R., Ojha, R., Lalitha, V., Raveesha, K.A. (2011). Antibacterial and Antioxidant Activity of CoffeabenghalensisRoxb.ExSchult.Fruit against Human Bacteria. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2(3): 856-865.
18. Malpani, M.O., Rajput, P.R., Mane, V.D., Deshpande, A.R. (2012). Phytochemical Screening, Characterization and In Vitro Antimicrobial Activity of Butea Monosperma Flower, Leaves and Gum: Methanolic and Aqueous Extract. Int J Chem Res., 3(1): 17-20.
19. Madhavan, S.A. (2021). GC–MS Analysis OfBioactive Compounds Present In ethanolic Leaf Extract AcalyphaIndica. Asian Journal of Advances In Research, 6(4): 16-22.
20. Manandhar, S., Luitel, S.,Dahal, R.K. (2019).In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J. Trop. Med., 2019: 1895340-5.
21. Mickymaray, S. (2019).Efficacy and Mechanism of Traditional Medicinal Plants and Bioactive

Compounds against Clinically Important Pathogens. Antibiotics, 8: 257.

22. Mohan, S.C.,Dinakar, S.,Anand, T., Elayaraja, R., SathiyaPriya, B. (2012).Phytochemical, GC-MS analysis andantibacterial activity of a medicinal plant Acalyphaindica. Int. J. Pharm. Technol. Res., 4(3): 1050-1054.