PHYHTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY EVALUATION IN *ACALYPHA INDICA* LEAF EXTRACTS.

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ABSTRACT

Acalypha indica is a traditionally used medicinal plant. As per the literature review it has been proven to be used for curing various ailments. The present study aims to estimate the different phytochemical composition of acalypha indica leaf extracts and as well as screen the plant extracts for their antimicrobial and antioxidant activity estimation. The results obtained have shown that the hexane followed by methanol extracts had the highest antioxidant, antimicrobial and anti-inflammatory potential. Further cell line studies would help in validation of the plants cytotoxic properties.

Keyword: *acalypha indica, phytochemical screening, antimicrobial property, antioxidant activity*.

1. INTRODUCTION

Acalypha indica (English: Indian acalypha, Indian nettle, three-seeded mercury) is an erect, simple or branched, slightly hairy annual herb growing upto 40-75 cm tall with ovate leaves. Flowers are green, unisexual found in catkin inflorescence. (1,2)

It occurs throughout tropical Africa and South Africa, in India and Sri Lanka, as well as in Yemen and Pakistan. It has possibly been introduced elsewhere as a weed. *Acalypha indica* occurs widely throughout the tropics of the Old World. In Africa it occurs in Nigeria in West Africa and further widely throughout tropical Africa and the Indian Ocean islands. It also occurs in India, South East Asia, and Oceania. It has been introduced to areas of the new world with favorable climates.

In West Africa the leaves are cooked and eaten as a vegetable. It is also browsed by cattle. In West and East Africa the plant is used as a medicinal plant. This plant is held in high esteem in traditional Siddha medicine as it is believed to rejuvenate the body. It has been used in traditional medicine for curing various ailments like skin diseases, rheumatoid arthritis, etc. This plant has been used majorly for wound healing by tribal people.

Based on the literature survey, the present study aims to study the phytochemical compositions of *acalypha indica* and test its antimicrobial and antioxidant potential. (3,4)

2. MATERIALS AND METHODS:

The plant material was collected and leaves were shade dried, made into fine powder. 250 ml of solvent was added to about 10 gms of dried powder for performing soxhlet extraction. The different solvents used for extraction were methanol, ethyl acetate, petroleum ether and hexane. The extraction was carried out for 48 hrs.

The extract obtained has been rotary evaporated and stored for further studies.

2.1 Phytochemical screening:

The obtained plant extracts were screened for their phytochemical properties using various qualitative tests. Alkaloids were tested using mayer's and wagners test, carbohydrates using Benedict's and fehling's test,

phytosterols using Salkowski's Test, phenols using ferric chloride test , flavonoids using alkaline reagent and lead acetate test and amino acids using ninhydrin test. (5,6)

2.2 Antioxidant activity estimation:

The antioxidant activity has been estimated using potassium ferri cyanide test.10 test tubes were taken and to each 2.5ml of 0.2M phosphate buffer (pH 6.6) was added. Then 2.5ml of 1% potassium ferricynide was added to all test tubes. To 10 eppendorf tubes volumes of 10µl, 20µl to 100µl of plant extract was added to tubes individually and volume was made up to 1ml by adding respective solvent. This makes a total 1ml of plant extract. Then this each 1ml plant extract in 10 different eppendorf tubes were added to respective test tubes and reaction mixture was incubated for 20mins at 50°C.Then 2.5ml of TCA was added to all tubes and centrifuged at 3000rpm for 10 minutes. After centrifuging, 2.5ml of supernatant liquid was collected and 2.5ml of distilled water and 0.5 ml of FeCl3 was added to all test tubes. UV absorbance was recorded at 770nm. (7, 8)

2.3 Antimicrobial activity estimation:

The antimicrobial activity was estimated for the different solvents extracts using disc diffusion assay against species bacillus, streptococcus, Pseudomonas and E-coli. The zones of inhibitions were calculated. (9,10)

3. RESULTS AND DISCUSSION:

The phytochemical analysis results are as shown in the following table 1.

Table -1 Phytochemical screening of different solvent extracts of *Acalypha indica* leaves

Test	Phytochemicals	Methanol extract	Ethyl acetate extract	Petroleum ether extract	Hexane extract
Mayer's test	Alkaloids	++	++	+	+++
Wager's test	Alkaloids	++	++	++	++
Benedict's test	Carbohydrates	-	-	-	-
Fehling's test	Carbohydrates	-	-	-	-
Salkowski's test	Phytosterols	-	-	-	-
Ferric chloride test	Phenols	++	-	-	-
Foam test	Saponins	+	++	-	-
Alkaline Reagent Test	Flavonoids	++	-	-	+++
Lead acetate test	Flavanoids	-	+	+	-
Ninhydrin test	Amino acids	+	+++	-	+

The antioxidant activity has been shown in the following table 2.

Table -2 Antioxidant activity estimation of different solvent extracts of *acalyphaindica* leaves:

Concentration	O.D Values of Methanolic extract	O.D Values of Ethyl acetate extract	O.D Values of Petroleum ether extract	O.D Values of Hexane extract
10µl	0.422	0.325	0.180	0.321
20µl	0.485	0.386	0.250	0.479
30µl	0.523	0.401	0.275	0.582
40µl	0.611	0.432	0.321	0.611
50µl	0.687	0.58	0.389	0.684
60µl	0.729	0.649	0.431	0.729
70µl	0.791	0.689	0.521	0.791
80µl	0.899	0.702	0.589	0.836
90µl	0.947	0.825	0.701	0.893
100µl	1.012	0.893	0.743	0.979

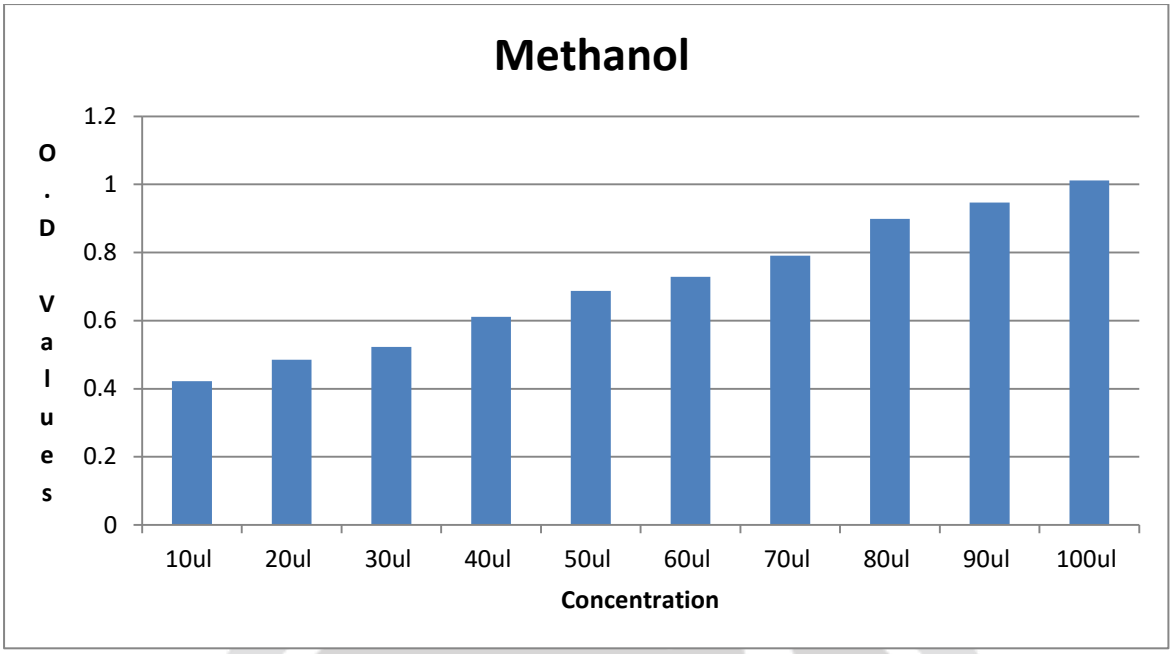


Chart -1: Antioxidant activity of methanol extracts

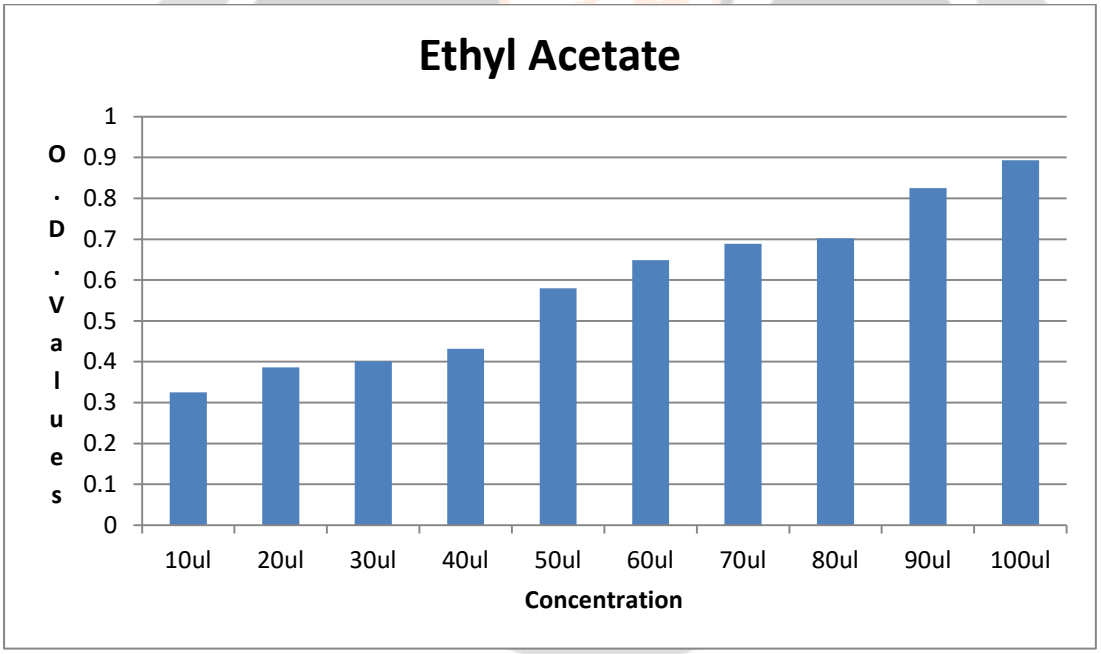


Chart-2: Antioxidant activity of ethyl acetate extract

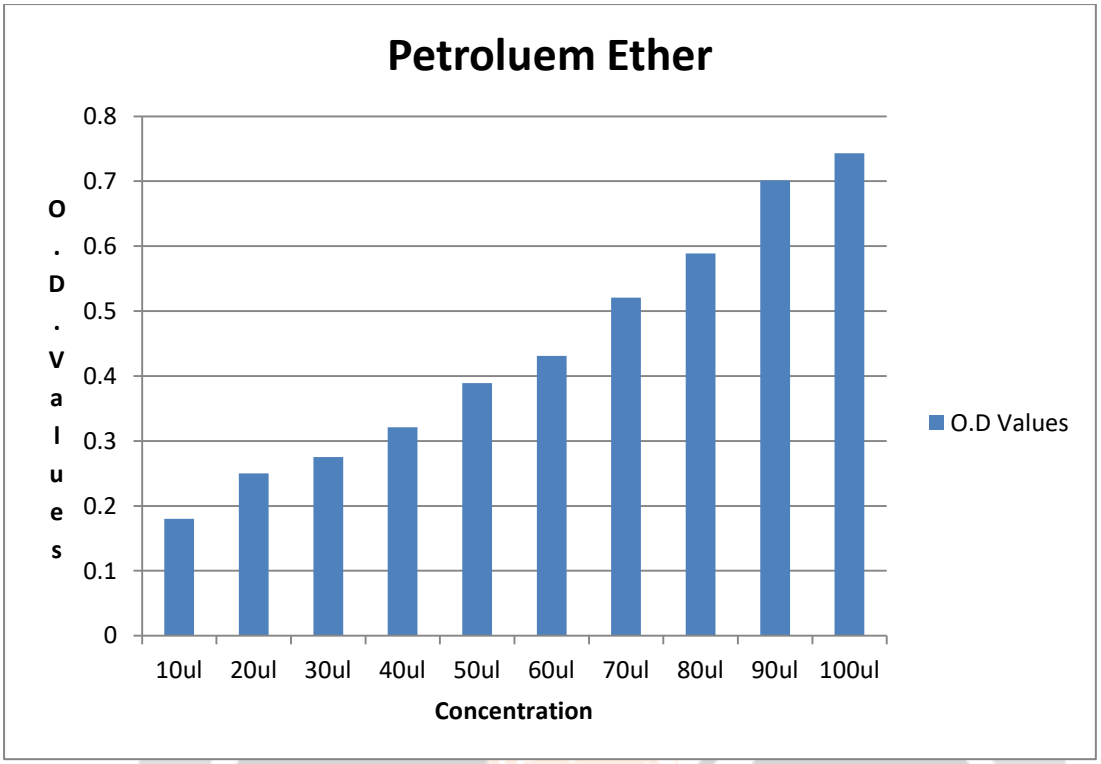


Chart-3: Antioxidant activity of petroleum ether extracts

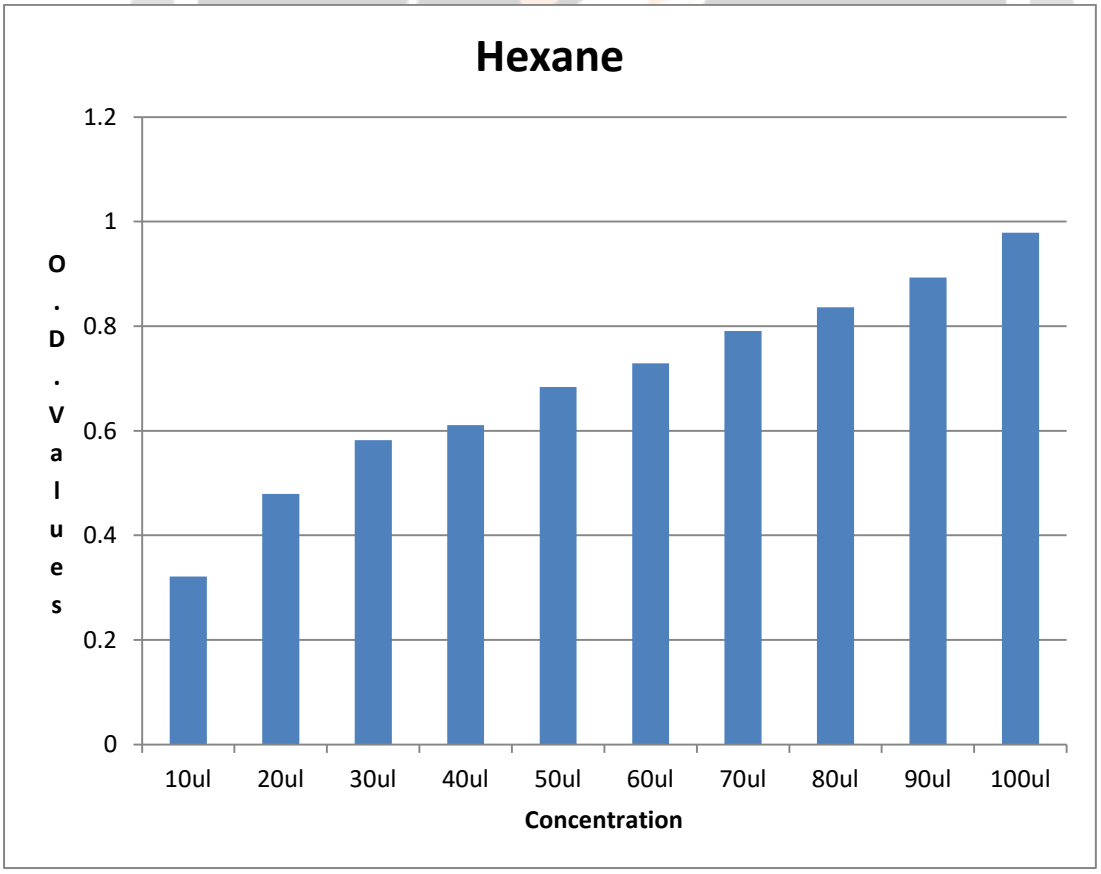


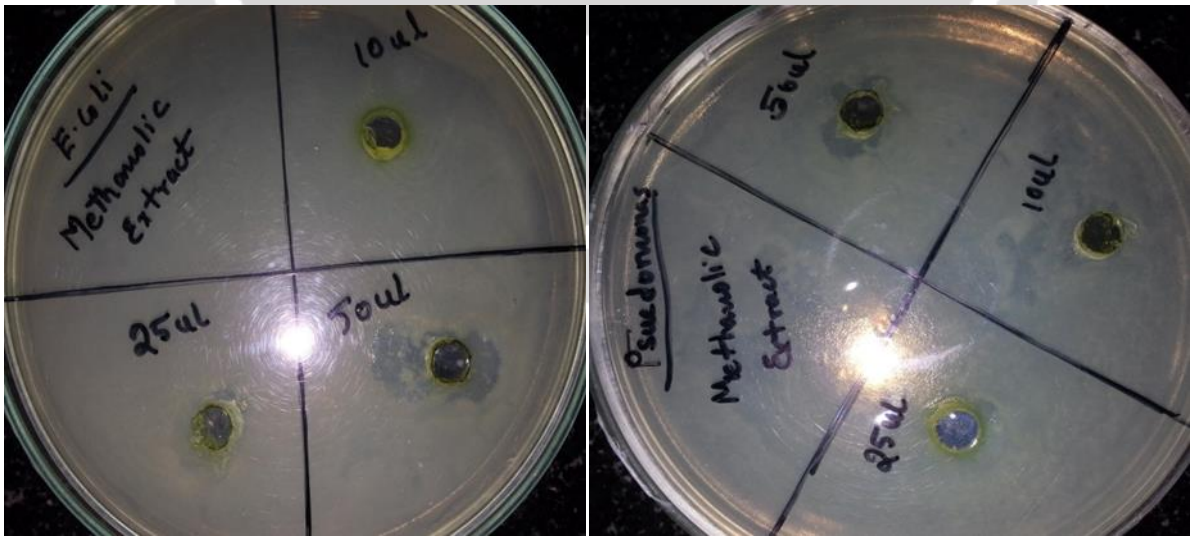
Chart-4: Antioxidant activity of hexane extracts

Antimicrobial activity:

The following table gives the different zone of inhibitions calculated from the disc diffusion assay against the species bacillus, e-coli, streptococcus and pseudomonas.

Table -3: Zone of inhibitions of different solvent extracts for antimicrobial activity:

Microbial Culture	Concentration Of Plant Extract	Zone Of Inhibition for methanol extract (In Mm)	Zone Of Inhibition for hexane extract (In Mm)	Zone Of Inhibition for petroleum ether(In Mm)	Zone Of Inhibition for ethyl acetate extract(In Mm)
Bacillus sps	50µl	6±1	5±1	2±1	5±1
	25µl	4±1	5±1	1±1	3±1
	10µl	2±1	2±1	1±1	1±1
E.coli	50µl	7±1	5±1	2±1	4±1
	25µl	4±1	4±1	2±1	3±1
	10µl	3±1	1±1	1±1	1±1
Psuedomonassps	50µl	4±1	4±1	3±1	4±1
	25µl	2±1	3±1	1±1	2±1
	10µl	1±1	1±1	1±1	1±1
Streptococcus sps	50µl	4±1	5±1	2±1	4±1
	25µl	4±1	3±1	1±1	1±1
	10µl	1±1	1±1	1±1	1±1



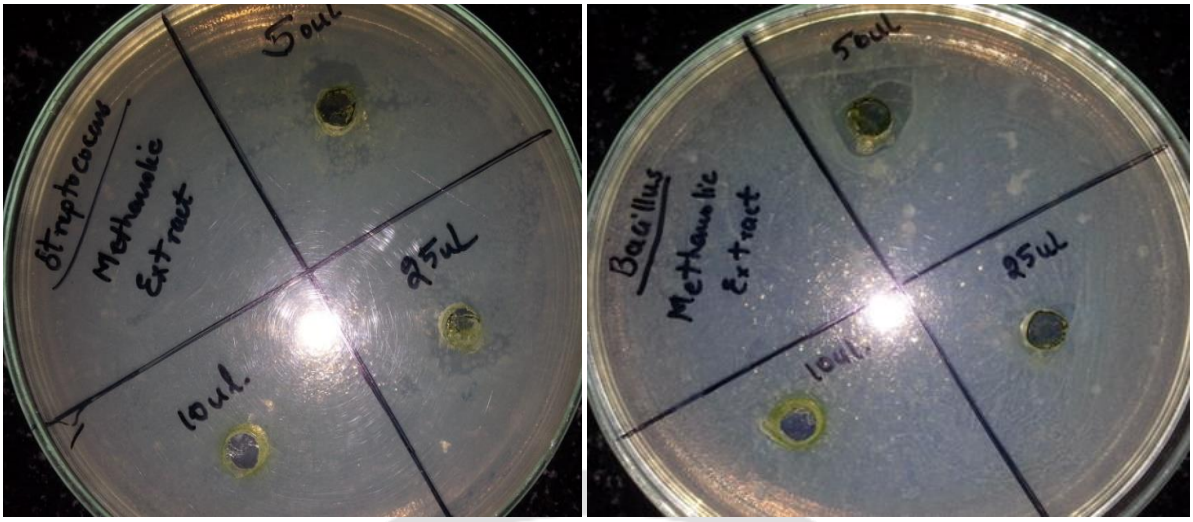


Fig-1: Zone of inhibitions formed for different bacterial cultures

4. DISCUSSION

Phytochemical analysis shows that most of the phytochemicals got dissolved in hexane and methanol followed by ethyl acetate and petroleum ether. A particular photochemical has its own affinity to a particular solvent. In the above result hexane has high affinity towards alkaloids and flavanoids and low affinity towards aminoacids. Ethyl acetate has high affinity towards aminoacids and good affinity towards alkaloids and saponins. Methanol has a good affinity towards alkaloids, phenols and flavonoids and low affinity towards saponins and aminoacids. Petroleum ether has a good affinity towards alkaloids and low affinity towards flavonoids. The phytochemical constituent which is common in all the 4 solvents are alkaloids. Antioxidant activity was performed for the extracts from 4 solvents. Absorbance value is highest for methanol extract followed by hexane, ethyl acetate and petroleum ether. This shows high antioxidation capacity for methanol extract. Higher the absorbance values, higher the antioxidation capacity. Anti-microbial activity for the extracts of methanol extract showed better results than to that of hexane, ethyl acetate and petroleum ether. A highest value of zone of inhibition was found in methanol extract against *E.coli.*

5. CONCLUSION

The medicinal properties of plants could be antioxidant, antimicrobial, anti-inflammatory based on their phytochemical compositions. In the present study, phytochemical analysis shows that the most of important plant phytochemicals were effectively dissolved in methanol and hexane followed by ethyl acetate and petroleum ether. Antioxidant work shows that the maximum antioxidation capacity is with methanol extracts followed by hexane, ethyl acetate and petroleum ether. Hence for antimicrobial activity methanol and hexane have been analyzed as these two solvents shown best results. Methanol has shown highest value of zone of inhibition against *E.coli* followed by *Bacillus*. Hexane has shown highest value of zone of inhibition against *E.coli* followed by *Streptococcus.*

The high antioxidant potential and rich phytochemical composition of the acaalypha plant extracts suggest it to be a potent source of cytotoxic material which could be further studied for application in cancer therapy. Further cell lines and in vivo studies would help in the validation of the present research work.

6. REFERENCES

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