Evaluation of Antioxidant & Antimicrobial Activity of the Plant *Acalypha indica*

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Abstract: ***Acalypha indica is an erect annual herb with numerous long branches covered with soft hairs. According to the Siddha text, 'Pathartha Guna Chinthamani' (Page no: 179),*** **In the present study the plant was subjected to phytochemical evaluation and anti-microbial activities against some Gram +ve and –ve microorganism. The total phenolic and flavonoid content indicates that these compounds are likely to be the main nociceptive and inflammatory contributing to the observed activities. Maximum phenolic 37.1 ± 1.34a(mg GAE/g) and flavonoid (39.91 ± 1.06 QE/g) contents were also found in the methanol extract. results showed that the highest antioxidant activity with IC50 values of 0.051 and 4.18 mg/mL for DPPH and ABTS, respectively.**

Index term- Acalypha indica, **anti-microbial, antioxidant activity, DPPH**

1.INTRODUCTION

Herbal drugs are the which are usually obtained from the plant source. It has been found that every plant in the universe has some kind of medicinal activities due to the presence of white variety of medicinal constituents. That is carbohydrates, 14 amino acids, lipids, tannin, alkaloids, saponin, flavonoids etc. A successful Isolation of this constituent may possess a wide variety of pharmacological activities. Same kind of medicinally active compound can be obtained through synthetic methods, for obviously natural compounds are little less harmful to any living body. Herbal medicines as compared to a synthetic pharmaceutical have great advantage.[1]

The use of herbal extracts and nutritional supplements, either as alternative or complementary medicine to the conventional chemotherapy for the treatment of inflammatory disease is well documented in Ayurveda, which is an alternative medicinal system that has been practiced primarily in the Indian subcontinent for 5000 years. The acceptance and recognition of herbal medicine is due to the acknowledgement of the value of traditional and indigenous pharmacopoeias, incorporation of some medicines derived from these sources into pharmaceuticals. The need to make health care affordable for all, and the perception that pharmaceuticals drugs are increasingly over prescribed, expensive, even dangerous. Another important perception fomenting this interest is that natural remedies ae somewhat safer and more efficacious than remedies that are pharmaceutically derived.[1,2]

Acalypha indica is one among them, and it is frequently used to cure a variety of diseases. Besides of India, it also occupied around Asia and African countries etc., to treat diverse health issues. More importantly, available information on this plant has not been reviewed completely previously published reviews. Hence, medicinal importance of this plant was made an attraction to review the available information for the asset to scientific community.[3]

Medicinal plants include several phytochemicals, including flavonoids, alkaloids, tannins, and terpenoids. These compounds have antibacterial and antioxidant capabilities. Extensive study has been conducted on the antibacterial properties of several plant species. For instance, the unrefined extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs demonstrate antibacterial characteristics against a broad spectrum of Gram-positive and Gram-negative bacteria. Furthermore, there have been reports indicating that extracts derived from Chinese chives and cassia had the ability to significantly inhibit the proliferation of Escherichia coli and other germs when meat, juices, and milk are stored. In a parallel investigation, examined the impact of different botanical extracts on the proliferation of Candida albicans. The findings revealed that the alcoholic extract derived from curry leaves

significantly impedes the growth of C. albicans, yielding a mean inhibition rate of 24.05 ± 0.07 after 48 hours. Furthermore, a study indicated that thyme oil extract has the ability to inhibit the growth of both C. albicans and Pseudomonas aeruginosa.[4,5]

1.1Plant Profile[13]

(Fig. 01 *Acalypha indica*)

1.2 Scientific classification

KINGDOM	PLANTAE
ORDER	Malpighiales
FAMILY	Euphorbiaceae
GENUS	Acalypha
SPECIES	indica

1.3 Vernacular names

LOCAL NAMES	COUNTRY
Muktajhuri	Bangladesh
Alcalifa	Brazil
Tie Xian	China
Kuppaimeni	India
Ricinela	Spain
Mukta barshi jhar	Nepal
Haan Maeo	Thailand
Tai Tuw Owjng Aasn	Vietnam

1.4 Pharmacological Properties

- Anti-Venom properties
- Antiulcer activity
- Cardio protective property
- Anthelmintic activity
- Antiarthritic activity
- Antifungal activity
- Anti-tuberculosis activity
- Wound healing property
- Analgesic activity
- Ovicidal activity
- Antibacterial Activity
- Antioxidant Activity
- Anti-inflammatory Activity
- Post-coital Infertility Activity

2.MATERIALS AND METHODS

2.1Collection, authentication and preparation of plant materials[6]

The plant *A. indica* was collected from Bargarh, Odisha, and identified by Botanist Sambalpur University, Sambalpur and the reference was deposited for the future reference. The plants were dried under shade, powdered and stored in an air tight container.

2.2Extraction of plant materials by Soxhlet extractor using solvent[5]

The dried plant of the *A. indica* were defatted with petroleum ether (60-80 °C) to remove waxy material, followed by extraction with ethanol in Soxhlet apparatus and concentrated using rotary evaporator at 40 °C. The percentage yield was recorded and the dried extracts stored in the refrigerator (4 °C).

2.3Phytochemical screening[10]

Qualitative phytochemicals study of the extracts was undertaken according to standard procedures.

Alkaloids

The extract was treated with HCl and filtered. To the filtrate various reagents are treated.

Mayer's test

To 2 ml of filtrate, Mayer's reagent was added. The presence of alkaloids was indicated by formation of white precipitated.

Wagner's test

To 2 ml of filtrate, Wagner's reagent was added. The presence of alkaloids was indicated by formation of reddish-brown precipitate.

Hager's test

To 2 ml of filtrate, Hager's reagent was added. Formation of yellow precipitate, indicating the presence of alkaloids.

Dragendorff's test

To 2 ml of filtrate, Dragendorff's regent was added. Formation of yellow precipitate, indicating the presence of alkaloids.

Carbohydrates and Glycosides

The extract was dissolved in water, filtered and the filtrate was used for various test.

Molish's test

Two ml of filtrate was taken and two drops of α-napthol (alcoholic solution) was added to it and vigorously shaken. To this solution one ml of concentrated H2SO4 was mixed slowly and allowed to stand. After that a violate colored ring was obtain that showed existence of carbohydrate.

Fehling's test

On water bath the filtrate, Fehling solution A and B was boiled. Red precipitate indicating the presence of sugar.

Barfoed's test

On water bath, the filtrate and Barfoed's reagent was boiled. Crimson precipitate indicating the presence of sugar.

Benedict's test

Benedict's reagent and filtrate was heated on water bath, dark brown precipitate was obtained indicating sugar present.

Glycosides

Fifty milligrams of extract were hydrolyzed with conc. HCl for two hours on a water bath and then sieved it. Then the hydrolysate was given below

Borntrager's test

Hydrolysate 2 ml of filtrate and to it 10 ml of chloroform was added then it shakes properly. After that the chloroform layer separated. The 10% of NH4 solution was added. The observation was found as pink colour which indicate the presence of glycoside.

Saponin

Distilled water was mixed with fifty milligram of plant extract and volume was made up to 20 ml and shacked in a cylinder (graduated) for fifteen minutes. Foam was observed due to presence of saponin.

Proteins and Amino Acids

Two mg of plant extract sample was poured and dissolved in 10 millilitre of water (distilled) and filter it through filter paper (Whatman's). The solution was allowed to test for protein and amino acid.

Millon's test

To the filtrate, Millon's reagent was added. A white precipitate was obtained which indicate the presence of proteins.

Phenolic Compounds and Tannins:

Ferric chloride test

Five ml of water (distilled) and fifty mg of plant extract were dissolved then added 5% neural ferric chloride solution resulted dark green colour that implies phenolic compound present.

Lead acetate test

Fifty milligrams of plant sample were mixed with water (distilled) and added 10% solution of lead acetate. White precipitate was formed, results showed presence of phenolic compound.

Alkaline reagent test

10% ammonium hydroxide sol. was treated by an aqueous solution of extract. Presence of flavonoids was showed by a yellow fluorescence.

Phytosterols

Solkowski test

The filtrate was taken in a test tube, chloroform and conc. H2SO4 was added. A brown colour ring was formed in sulphuric acid layer, which showed the presence of phytosterols.

2.4 Pharmacological screening[10,11,12]

2.4.1 Antimicrobial Screening

Screening for antibacterial activity of the extracts was done by agar disk diffusion method followed by determination of minimum inhibitory concentration (MIC) of the shortlisted active plant extracts against the bacterial strains. Antimicrobial activity was determined against standard bacterial strains.

Material used in the agar diffusion assay were Biological safety cabinet, bacteriological incubator, semi-micro analytical weighing balance, cork borer, suction apparatus with silicon tubings (Borosil), micropipettes, Vernier callipers; Mueller Hinton agar, cation adjusted Mueller Hinton broth, Sabouraud's dextrose broth and agar, 0.85% sodium chloride solution (physiological saline), sterile water, dimethyl sulfoxide; extraction solvents; test tubes, beakers, conical flasks and other glass apparatus; 150mm and 90mm polystyrene/polypropylene Petri dishes, polystyrene/polypropylene tips and microtips; ciprofloxacin, and plant extracts. The microbial stain was used Escherichia coli.

The procedure of antimicrobial disk diffusion method was; Mueller Hinton agar (MHA) and Sabouraud's dextrose agar (SDA) were used for preparation of the assay plates for the bacteria and yeasts respectively. Overnight grown microbial cultures from MHA and SDA plates were used to prepare the inocula of the bacteria and yeasts respectively. Several similar looking colonies were picked from the 82 culture plates and suspended in cation adjusted Mueller Hinton broth or Sabouraud's dextrose broth (SDB) accordingly for bacterial strains. Turbidity of the suspensions were adjusted to 0.5 McFarland using Densi-La-Meter or Densimat. Prepared microbial inocula were seeded in or swabbed on the surface of the media agar plates. 200 µL, 400 µL, 600 µL, 800 µL of plant extracts (in all their extraction solvents) were dispensed in the wells.

Plates were incubated at 35°C-37°C in the incubator for 18-24 hrs for bacteria and 24- 48 hrs for yeasts. Antimicrobial activity was observed as the zone of inhibition of microbial growth around the wells (diameter in mm) which was measured using the vernier callipers. All the plant extracts were tested against standard Gram positive & Gram-negative bacteria. Antibacterial drugs ciprofloxacin (25 µL, 50 µL, 75 µL, 100 µL) was included as the standard drug.

Antioxidant activity[10,13,14,15]

2.5.1Total phenolic content

The Gallic acid equivalence method (GAE), uses a mixture of phosphomolybdate and phosphotungstate for the colorimetric assay of phenolic and polyphenolic antioxidant.

Reagents used

Dilute folin ciocalteu reagent with equal volume of distilled water, 20 % of sodium carbonate in water, gallic acid.

Procedure

The preparation was made the calibration curve of standard gallic acid 10-100 µg/ml in water. The test solution was made 1 mg/ml. The sample was mixed with 0.25 ml of folin ciocalteu reagent and 1.25 ml of 20% sodium carbonate solution. The sample was incubated for 40 minutes at room temperature, after that the samples were compared with standard at 725 nm.

Calculation

The number of total phenols from calibration curve as a Gallic acid equivalent by the formula; T = C. V. / M

2.5.2. Total flavonoid content

The basic principle of Aluminium chloride colorimetric method is that Aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C- 5 hydroxyl group of flavones and flavonols.

Reagents used

Quercetin, ethanol, aluminium chloride, potassium acetate.

Procedure

The standard solutions were made 10-100 µg/ml concentration in methanol and the preparations were mixed with reagents. Incubated for 45 minutes in a dark place. The test solution was prepared 1mg/ml concentration and was mixed with the reagents (incubated for 45 minutes in a dark place). The test sample was compared with the standard with 415nm.

2.5.3.DPPH Radical Scavenging Activity

The free radical scavenging property of *A. indica* were investigated by DPPH assay. Ascorbic acid was used as standard. The percentage inhibition was calculated by using following formula:

% of Inhibition =A-B/B*100

Where, A is the absorbance of control, B is the absorbance of sample at different concentration.

All the assay methods were performed in triplicate.

Determination of IC50

A calibration curve drawn from percentage inhibition against concentration was used to determine the IC50 values of extracts.

2.5.4 Statistical analysis

The data were analyzed by both MS EXCEL (version 2019) and SPSS (version 21, IBM corporation). The data of anthelmintic activity were reported as mean ± standard deviation and the significant level P values less than 0.01, 0.05 and 0.001 were considered as statistically significant.

3.RESULTS AND DISCUSSION

3.1.Extraction of plant materials by Soxhlet extractor using solvent

The shade dried powder plant material was extracted by the help of solvent with increasing order of their polarity. The result of the extractive value possesses. The extractive values were reported in the Table 01.

Table 3.1: Percentage yield of Extracts

Sl. No.	Solvent used	Percentage yield
		A. indica
1	Water	2.25
2	Ethanol	5.5
3	Methanol	7.8
4	Acetone	1.5
5	n-Hexane	0.25

3.2Phytochemical screening

Preliminary phytochemicals screening of plant extracts:

Qualitative phytochemical investigations were conducted and the results were tabulated (Table 02).

Table-3.2: Phytochemicals test of *A. indica*

Sl.no	Phytochemical test	*A. indica*
		Methanolic
1. Alkaloids		
a	Mayer's test	+ve
b	Wagner's test	-ve
c	Hager's test	-ve
d	Dragendorff's test	-ve
2. Carbohydrates & Glycosides		
a	Molish's test	+ve
b	Fehling's test	-ve
c	Barfoed's test	+ve
d	Benedict's test	-ve
e	Borntrager's test	+ve
3.Saponins		
a	Foam test	
4. Proteins & amino acid		
a	Millon's test	+ve
5. Phenolic compounds & flavonoids		
a	Ferric chloride test	+ve
b	Lead acetate test	+ve
c	Alkaline test	+ve
6. Phytosterol		
a	Solkowski test	+ve
+ve, Present; -ve, Absent		

3.3Pharmacological screening

Antimicrobial activity:

The methanolic extract of *A. indica* showed the weak anti-microbial activity against E. coli bacteria. The zone of inhibition of methanolic extract of *A. indica* was 3 mm at 800 µg (Table 3.3& Fig1-4).

Table 3.3: Antibacterial activity of methanolic extract of *A. indica*

Treatment	Dose (µg/ml)	Zone of inhibition (mm)			
		E. coli (Gram - ve)	*S. typhi* (Gram - ve)	*P.aeruginosa* (Gram -ve)	*S. aureus* (Gram+ve)
Negative Control (DMSO+ microorganism)	200 µl	0	0	0	0
	400 µl	0	0	0	0
	600 µl	0	0	0	0
	800 µl	0	0	0	0
Positive Standard (Ciprofloxacin + microorganism)	25 µg	0	20 ± 0.13	0	08 ± 0.19
	50 µg	08 ± 0.14	20 ± 0.36	07 ± 0.29	09 ± 0.14
	75 µg	11± 0.29	21 ± 0.32	07 ± 0.51	13 ± 0.22
	100 µg	16 ± 0.11	22 ± 0.18	09 ± 0.23	15 ± 0.16
Methanolic Extract (Test-1)	200 µg	03 ± 0.32	02 ± 0.34	04 ± 0.39	03 ± 0.29
	400 µg	04 ± 0.22	03 ± 0.44	06 ± 0.29	05 ± 0.49
	600 µg	06 ± 0.22	05 ± 0.44	08 ± 0.29	06 ± 0.49
	800 µg	07 ± 0.26	06 ± 0.44	09 ± 0.25	08 ± 0.41

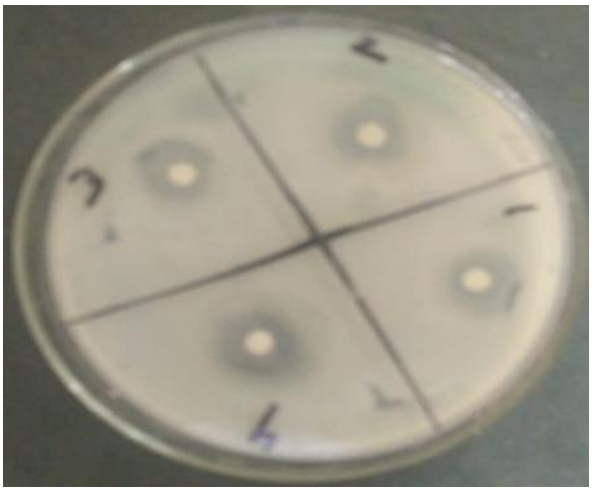


Figure 3.1 *E. coli*

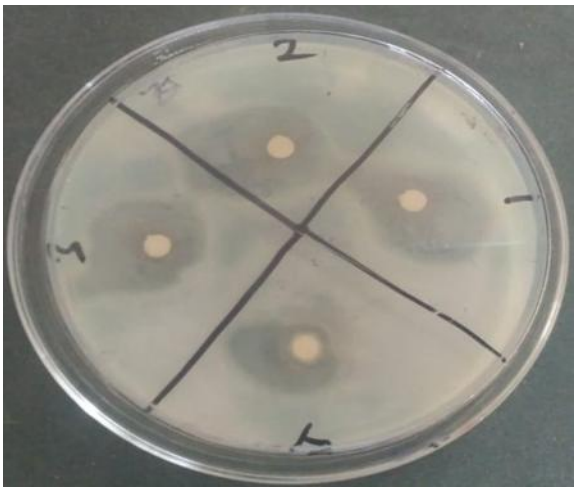


Figure 3.2 *S. typhi*

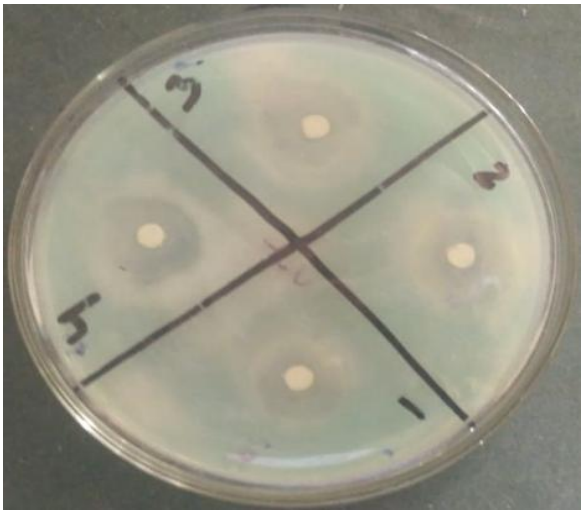


Figure 3.3 *P.aeruginosa*

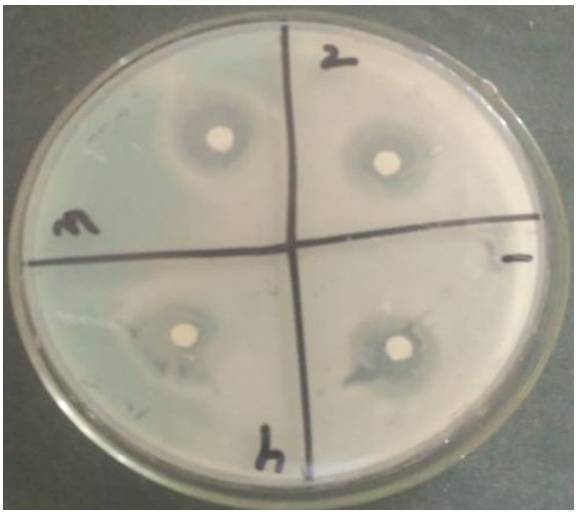
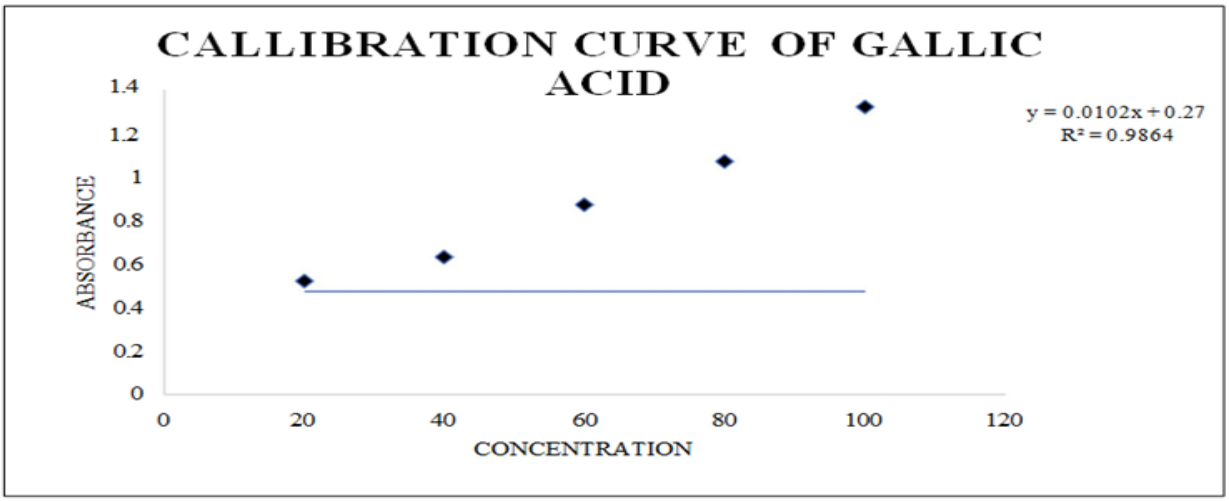


Figure 3.4 *S. aureus*

Antioxidant activity:

Total phenolic content of methanolic extracts of *A. indica*

Total phenolic content of methanolic extract of *A. indica* was found as 37.1 ± 1.34 mg GAE/g (Table 3.4 Fig-3.5).



3.5: Calibration curve of Gallic acid for total phenol content determination

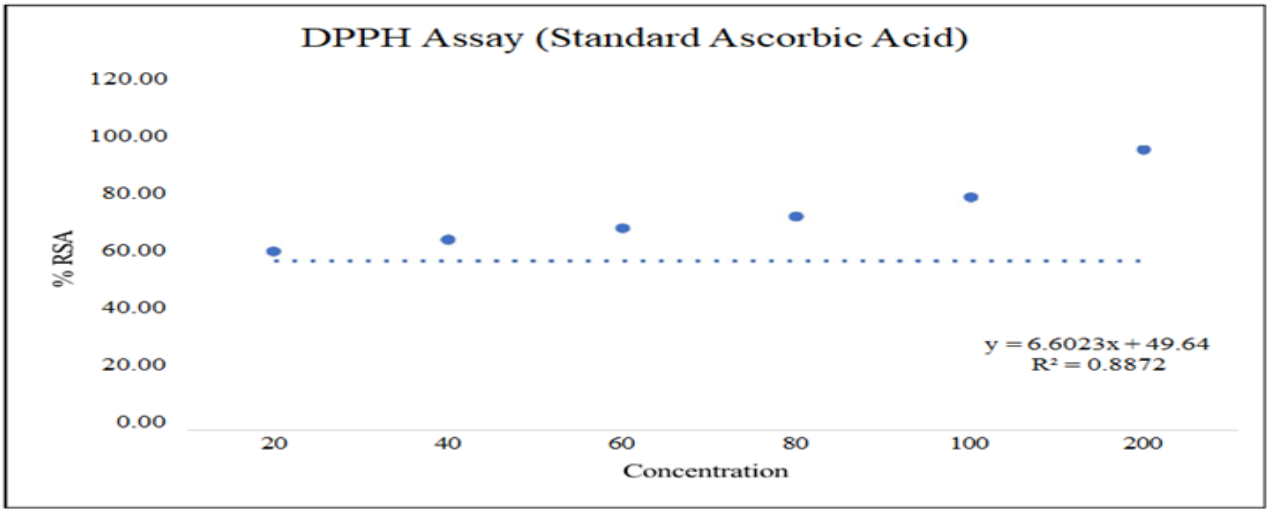
Table 3.4: Total phenolic contents of methanolic extracts of *A. indica*

Sample	TPC (mg GAE/g)
Methanolic Extract	37.1 ± 1.34

GAE: Gallic acid equivalentValues are expressed as mean ± SEM, n=3 and was estimated by One-way ANOVA followed by Dunnet test. *p<0.05 considered as significant.

Total Flavonoid content of methanolic extracts of *A. indica:*

Total flavonoid content of methanolic extract of *A. indica* was found as 39.91 ± 1.06 mg QE/g (Table 3.5 & Fig. 3.6).



3.6: Calibration curve of Gallic acid for total Flavonoid content determination

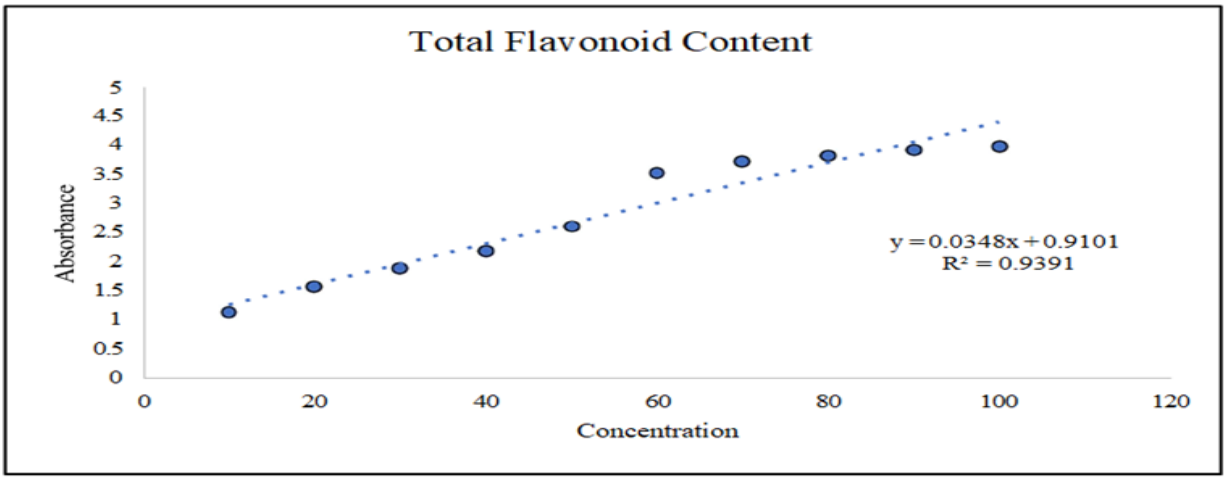
Table 3.5: Total Flavonoid contents of methanolic extracts of *A. indica*

Sample	TFC (mg QE/g)
Methanolic Extract	39.91 ± 1.06

QE: Quercetin equivalentValues are expressed as mean ± SEM, n=3 and was estimated by One-way ANOVA followed by Dunnet test. *p<0.05 considered as significant.

DPPH Radical Scavenging Activities of methanolic extracts of *A. indica:*

The antioxidant potential of *A. indica* at 20, 40, 60, 80, 100, and 200 µg/ml against free radicals was studied by DPPH. *A. indica* significantly scavenged the free radicals by DPPH method at highest concentration (64.41) with corresponding IC_{50} values of 4.18 respectively in comparison to standard ascorbic acid (Table 3.6 & Fig. 3.6).



3.6: DPPH Scavenging activity of Ascorbic acid

Table 3.6: Calculation of % Radical Scavenging and IC50 from DPPH Assay of Ascorbic Acid

Absorbance Measurement Data (STD Ascorbic Acid)				
Concentration µg/ml	Abs of Control	Abs of Sample	% RSA	IC50
20	0.222	0.09	59.46	0.05
40	0.222	0.081	63.51	
60	0.222	0.072	67.57	
80	0.222	0.063	71.62	
100	0.222	0.048	78.38	
200	0.222	0.009	95.95	

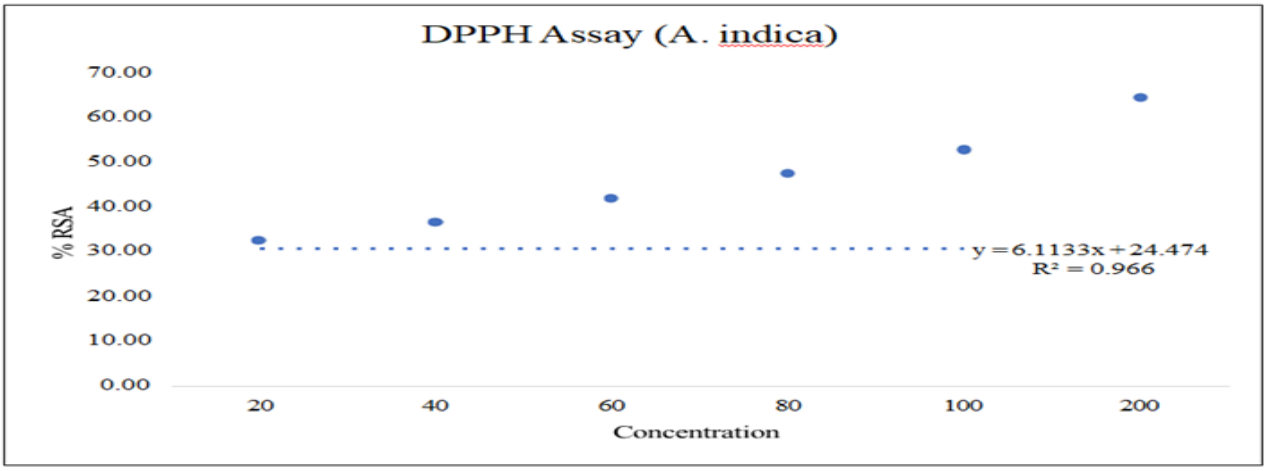


Fig. 3.7: DPPH Scavenging activity of methanolic extract of *A. indica*

Table3.7: Calculation of % Radical Scavenging and IC50 from DPPH Assay of methanolic extract of *A. indica*

Absorbance Measurement Data (*A. indica*)				
Concentration µg/ml	Abs of Control	Abs of Sample	% RSA	IC50
20	0.222	0.15	32.43	4.18
40	0.222	0.141	36.49	
60	0.222	0.129	41.89	
80	0.222	0.117	47.30	
100	0.222	0.105	52.70	
200	0.222	0.079	64.41	

4.CONCLUSION

On the basis of the findings of the study, one can draw the conclusion that the plant contains a variety of phytochemicals. The conclusion that can be drawn from the pharmacological findings is that all extracts of *A. indica* has antimicrobial and antioxidant activity when compared to conventional and standard drugs. It is necessary to do additional research utilizing in vivo models in order to determine and establish the efficacy of as well as the pharmacological reasoning for the utilization of leaves and the entire plant as an anthelmintic medicine.

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