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A Study on the Phytochemical analysis of *Tarlmouniaelliptica* (DC.) "H.Rob., S.C.Keeley, Skvarla&R.Chan"

[1] Bharathkumar A., [1*] Vijayaraj R., [2]Madeena Begam S., and [2*]JaqulineChinna Rani I.

[1]Department of Plant Biology and Biotechnology, Molecular and Microbial Biotechnology Unit & Loyola Institute of Frontier Energy (LIFE), Loyola College, Nungambakkam, Chennai - 600 034.

[2*]Assistant Professor, Department of Plant Biology and Biotechnology, Molecular and Microbial Biotechnology Unit & Loyola Institute of Frontier Energy (LIFE), Loyola College, Nungambakkam, Chennai - 600 034.

Corresponding author: bharatbio95@gmail.com

Abstract

Plants, acting as an essential source of nutrients and medicinal ingredients, have long been known for their natural arsenal. Human beings have used plants as medicinal items for around 600,000 years. The preparations released from certain plants lead to plant medicines. Today, plant medicines are popular worldwide, where herbs are used as primary medicines by around 80% of the world's population. This medicinal importance is primarily due to the richness of bioactive phytochemicals in plants.

In this study, we aimed at screening the phytochemicals and biological activities of *Tarlmouniaelliptica*(DC.) Fresh leaves, derived from plant material, were dissolved in four different solvents methanol, chloroform, benzene and distilled water. The phytochemical composition of extracts showed the presence of alkaloids, phenolics flavonoids and terpenoids. DPPH radical scavenging and Fe^{2+} chelating activity assays and Phosphomolybdenum assay were used to assess the antioxidant potential. The anti-inflammatory potential was evaluated by analyzing the Inhibition of albumin denaturation. Biological activity of extract such as anti-diabetic and anti-fungal activity was also tested and the result showed maximum activity of plant species.

Collectively, our observations suggest a promising potential for *Tarlmouniaelliptica*(DC.) during the treatment of diseases associated with oxidative stress, aberrant inflammatory responses, and glucose-sugar control. However, further experimental procedures are needed to support this postulate.

Keywords: Albumin, Amylase, Antioxidant, Denaturation.

1. Introduction

Plants have been used for medicinal purposes since human civilization. Research reports the use of herbs by Unani, Indian vaids, Europeans and Mediterranean cultures for more than 5000 years. Therefore, medicinal plants **(Han DR *et al.*, 1989)**are also called as medicinal herbs. Indigenous cultures such as Rome, Egypt, America and Africa used these herbs as ahealing ritual until the systematic use of these herbs was developed into the various traditional systems of medicine. On the account of side effects caused by synthetic drugs, resistance development to currently available drugs to treat diseases, excessive **(Akhtar H *et al.*, 1992)** cost of treatment, inadequate supply to rural developments and most importantly

high population value, the traditional system of medicine has proven to be adequately efficient and promising for the treatment and cure of various human diseases.

Recently, the WHO (World Health Organization) estimated that more than 70% of the world population fully depend on the medicinal herb as a therapeutic potential herb for their basic primary health care needs.

In the renaissance of medicinal plants, the plant *Tarlmouniaelliptica* (DC.) is used for wide variety of purposes with less or no research on the phytochemical and pharmacological activity of plant species. *Tarlmouniaelliptica*(DC.) kingdom of Plantae is a plant genus in the family Asteraceae native to India, Burma and Thailand. The common name of this plant is curtain creeper, vernonia creeper or parda bel. This plant is similar to the sunflower family also named Compositae, a wide-spread family of flowering plants. The *Tarlmouniaelliptica* (DC.)plant grows thicker and more lustrous with annual fertilizer and shearing. The wide range of this plant is used to cure allergy. The woods are also used for furniture, cabinet-work etc. The rich tradition **(Gadgiland Berkes, 1991)** of utilizing such a plant is yet to be exploited which calls for the high demand of research by scientists. The term Tarlmounia is also sometimes called *Vernonia elaeagnifolia*(DC.) *et al*.

2. Materials and Methods

2.1 Identification and authentification of plant material

Tarlmouniaelliptica(DC.)fresh plants were collected from Madhavaram in Thiruvalluvar district. The identification and authentification of the selected plant for current research work was carried out before its extraction.

2.2 Taxonomical Classification

Kingdom	:	Plantae
Family	:	Asteraceae
Genus	:	Tarlmounia
Order	:	Asterales
Species	:	T. elliptica

2.3 Processing of Plant Material

Each part of the plant was washed under running water to make it free from dust and foreign particles. The plant parts were powdered and kept in an airtight container before analysis.

2.4 Preparation of Extracts

50g of powdered sample was macerated in 150ml methanol, kept in the orbital shaker for 48 hours and filtered using the Whatman No.1 filter paper. The extract is transferred into Petri-plate and evaporated to dryness. The same procedure is successively repeated for chloroform, benzene and aqueous extracts.

3. Preliminary Qualitative Analysis

3.1 Test for alkaloids

a. Mayer's test:

Two drops of Mayer's reagent were applied to the sides of the test to a few ml of plant sample extract. The presence of alkaloids suggests the emergence of the white creamy precipitate.

b. Wagner's test:

A few drops of Wagner's reagent were applied to the sides of the test tube to several ml of plant extract. The test is confirmed by a reddish-brown precipitate as positive.

3.2 Test for Carbohydrates

a. Molish's test:

To 2ml of plant sample extract, 2 drops of alcoholic solution of alpha naphthol was added. The mixture was thoroughly shaken, and a few drops of Conc. H_2So_4 was slowly inserted into the sides of the test tube. The presence of carbohydrates is indicated by a violet ring.

3.3 Test for Glycosides

50mg of extract was hydrolysed with Conc. Hcl for 2 hours on a water bath, filtered and the hydrolysate was subjected to the following tests.

a.Borntrager's test

3ml of chloroform was applied to 2ml of filtered hydrolysate and shaken, the chloroform layer was removed and 10% NH_3 solution was added to it. The presence of glycosides is shown in pink.

b. Legal's test:

In pyridine, 50mg of extract was dissolved, sodium nitroprusside (SNP) solution was added and 10% alkaline was formed. The glycoside presence is indicated by the pink hue.

3.4 Test for Saponins

The extract (50mg) was diluted in distilled water and up to 20ml was formed. The suspension was shaken for 15 mins in a graduated cylinder. The presence of saponins suggests a 3cm layer of form.

3.5 Test for Proteins and Amino acids

a. Millon'stest:

A few drops of Millon's reagent were applied to 2ml of the filtrate. A white precipitate suggests that protein is present.

b. Biuret test:

With 1 drop of 2% $CuSo_4$ solution, 2ml of the filtrate was treated 1ml of ethanol (95%) is added to this, followed by excess pellets of potassium hydroxide (KOH). The presence of proteins is shown by the pink ethanolic layer.

3.6 Test for Amino acids

The extract (100mg) was dissolved in 10ml of distilled water and filtered through What manNo.1 filter paper and the filtrateis subjected to test for amino acids.

a. Ninhydrin test:

2 drops of ninhydrin solution were applied to 2ml of aqueous filtrate (10mg of ninhydrin in 200ml of acetone). The appearance of purple suggests that amino acids are present.

b. Benedict's test:

0.5ml of Benedict's reagent was added to 0.5ml of the filtrate. The mixture was heated for 2 mins in a boiling water pan. A distinctive colored precipitate shows the presence of sugar.

c. Fehling's test:

It was hydrolyzed and diluted from the extract. Hcl, neutralized with alkali and heated with the A and B solution from Fehling. Red precipitate formation suggests the presence of sugar reduction.

3.7 Test for Phenolic Compounds

a. Ferric chloride test:

In 5ml of distilled water, the extract (50mg) was dissolved. A 5% $FeCl_3$ solution has been applied to these few drops. The presence of phenolic compounds is indicated by a dark green color.

b. Gelatin test:

The extract (50mg) was dissolved in 5ml of distilled water and 2ml of 1% gelatin containing solution was added to it. The presence of phenolic compounds is indicated by White Precipitate.

c. Lead acetate test:

The extract (50mg) was dissolved in distilled water and a 10% lead acetate solution was added to this 3ml solution. The presence of phenolic compounds suggests a bulky white precipitate.

3.8 Test for Flavonoids

a. Alkaline reagent test:

A 10% NH_4OH solution was treated with an aqueous solution of the extract. Yellow fluorescence suggests that flavonoids are present.

b. Magnesium and hydrochloric acid reduction:

The extract (50mg) was dissolved in 5ml of alcohol and a few fragments of magnesium ribbon and Conc. Hcl (dropwise) were added. The existence of flavonol glucosides is inferred if any pink to crimson colour develops.

3.9 Test for Terpenoids

Few drops of plant extract and 2ml of chloroform was mixed well. To this mixture a little amount of Conc. H_2So_4 was added drop by drop,the formation of reddish-brown color indicates the presence of terpenoids.

3.10 Test for Steroids

a. Libermann-Burchard's test:

In 2ml of acetic acid anhydride, the extract (50gm) was dissolved. For this, one or two drops of Conc. H_2So_4 is applied progressively to the sides of the test tube. The presence of phytosterols is shown by a color shift array.

b. Salkowski test:

The extract was processed and filtered with chloroform. There were a few drops of Conc. H_2So4 treated with the filtrate. Shaken and allowed to stand, the appearance of the colour golden yellow suggests the presence of triterpenes.

4.Quantitative Estimation of Phytochemicals

4.1 Total phenol content determination:

100mg of sample extract was correctly weighed and dissolved in 100ml of triple distilled water (TDW). 1ml of this solution was transferred to a test tube, then 0.5ml 2N of the Folin-Ciocalteu Reagent and 1.5ml 20% of Na_2Co_3 solution were added and eventually up to 8ml of TDW volume was developed accompanied by intense shaking and finally allowed to stand for 2 hours after the absorption was taken at 765nm. These data were used to use a standard calibration curve obtained from different diluted gallic acid concentrations to estimate the total phenolic content.

4.2 Total flavonoid content determination:

The method is based on the flavonoid-aluminum complex formation, which has a maximum absorptivity at 415nm. 100µl of methanol plant extracts (10mg/ml) were combined with 100µl of 20% $AlCl_3$ in methanol **(Chang *et al.*, 2002)** and a drop of acetic acid, then diluted to 5ml with methanol. After 40 mins, the absorption was read at 415nm. From 100ml of plant extracts and a drop of acetic acid, blank samples were prepared **(Krishnaiah*et al.*, 2009)** and then diluted to 5ml with methanol. In the same conditions, the absorption of standard rutin solution (0.5 mg/ml) into methanol was measured. Both determinations have been made in triplicates.

4.3Total alkaloid content determination:

5g of the sample was weighed into a 250ml beaker and 200ml of 10% CH_3COOH in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Until the precipitation was complete, Conc. NH_4OH hydroxide was applied dropwise to the extract. The entire solution was allowed to settle and the precipitated solution was gathered and washed with dil. NH_4OH and filtered afterward. The alkaloid that was dried and measured is the residue.

4.4Total terpenoid content determination:

1ml of the extract was taken with 3ml of ethanol and then filtered. To the given filtrate, 2ml of 4% aqueous phophomolybdic acid solution and 2ml Conc. H_2So_4 was added and mixed thoroughly. The mixture was left for 15 mins and was taken at 700nm.

5.Antioxidant Analysis (Qualitative analysis)

5.1 DPPH radical scavenging assay:

The sample is allowed to react with DPPH radical in a methanol solution. The given mixture of variables **(Desmarchelier*et al*., 1997)** concentration of sample with 2ml of radical solution (0.4mm) is considered. This reaction was incubated for 20 mins in dark surroundings. Colour changes occurs (from deep violet to light yellow) which was read at 517nm using a UV-spectrometer mixture of methanol and sample work as blank.

$$\text{DPPH (\%)} = \frac{Absorbance\ of\ the\ blank - Absorbance\ of\ the\ antioxidant}{Absorbance\ of\ the\ blank} \text{ x } 100$$

5.2 Ferric reducing antioxidant power (FRAP):

Freshly prepared working FRAP reagent was pipetted using 10-50µl variable micro-pipette (3.995ml) and mixed with 1ml of the approximately diluted plant sample and mixed thoroughly. An intense blue colour complex **(Oyaizu, 1986)** was formed when ferric tripyidye triazine complex was reduced to Fe^{2+} form and the absorbance at 593nm was recorded after 15 mins incubation at 37°C. All the determination was performed in triplicates.

5.3 Phosphomolybdenum antioxidant assay:

It is a spectroscopic method for the quantitative determination of antioxidant capacity, 10µl of the sample solution is combined with 1ml of reagent (0.049g add ammonium molybdate, 0.036g of sodium phosphate, 0.588ml of H_2So_4 added with dis. H_2O) the test tube closed capped and incubation is carried out for 90 mins in water both for about a temperature of 95°C after the process incubation the solution **(Prieto, 1999)** mixture is allowed to cool at room temperature and measured at 695nm against blank in UV-spectrometer the same solvent is used for other/all samples and it is incubated under the same condition.

6. Anti-Inflammatory Assay

6.1 Egg albumin denaturation assay:

The reaction mixture (5ml) consisted of 0.5ml of egg albumin and 1.5ml of phosphate buffer, saline (PBS, pH 6.4) and inconstant concentrations (20µl, 40µl…..100µl) of the test extract and standard drug. The corresponding volume of PBS without drug provided as a control. Later the mixtures **(Mizushima and Kobayashi, 1968)** were incubated for 15 mins at 37±2°C and again incubated for 10 mins over 70°C. After cooling, their absorbance was surveyed, at 660nm using vehicle as blank.

$$\text{Percentage of inhibition (\%)} = \frac{Absorbance\ of\ the\ control - Absorbance\ of\ the\ sample}{Absorbance\ of\ the\ control} \text{ x } 100$$

7. Anti-Diabetic Assay

7.1 Inhibition of α-amylase enzyme:

15µl of the plant extract at different concentrations (50µl/ml – 200µl/ml) diluted in phosphate buffer was added to 5µl of enzyme porcine pancreatic solution into a 96-well plates. After 10 mins of incubation at 37°C, the reaction was initiated by adding 20µl of starch solution and further incubated for 30 mins at 37°C. The reaction was then **(Odeyemi, 2015)** stopped by adding 10µl of 1M Hcl to each well followed by 75µl of iodine reagent. A blank containing phosphate buffer (pH 6.9) instead of the extract and positive control (acarbose,

64µg/ml) were prepared. For such research samples, there was no enzyme control and no starch control. The absorbance was estimated at 558nm and the inhibitory activity percentage was determined.

$$\text{Percentage of inhibition (\%)} = \frac{Absorbance\ of\ the\ sample - Absorbance\ of\ the\ control}{Absorbance\ of\ the\ blank - Absorbance\ of\ the\ control} \text{ x } 100$$

8. Anti-fungal Activity

Potato dextrose agar (PDA) was prepared, potato (200g), dextrose (20g), and agar (20g) each were poured in sterile Petri-dishes with different species *Candida albicans* and *Aspergillus niger*. Five holeswere made using sterile corkborer and a total of 20µl extract and control 10µl was poured and there were incubated. The zone inhibition **(Wayne, 2000)** was identified. Later the readings were recorded. Clotrimazole and fluconazole were used as standard anti-fungal drugs.

9. Results and Discussion

Discovering and screening of potential phytochemical andpharmacologicalactivity from natural plant products is still the main scope for many of pharmaceutical and medical scientists. In the entire world, tremendous resources are being investigated in thediagnosis, prevention and treatment of human pathogens and diseases.

Phytochemical screening

Phytochemical screening tests for different extracts of *Tarlmouniaelliptica*(DC.) showed the active phytochemical classes as alkaloids, glycosides, proteins, phenolics, terpenoids, flavonoids and steroids as presented in Table 1.

Table1:

Phytochemical screening tests for the methanol, chloroform, benzene and aqueous*Tarlmouniaelliptica* (DC.)extracts.

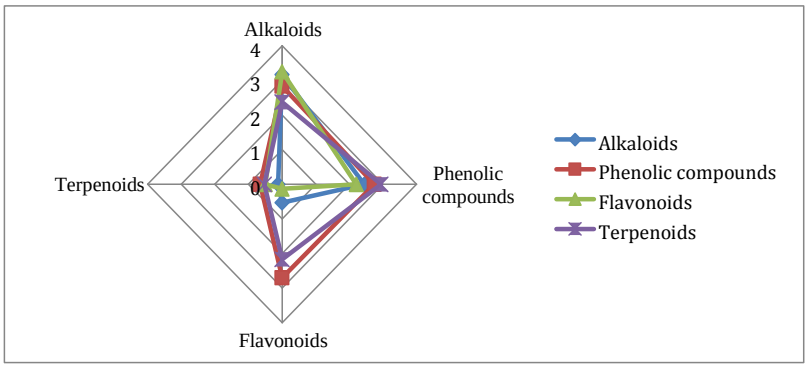
S.No	Phytochemical Constituents	Solvents			
		Methanol	Chloroform	Benzene	Aqueous
1	Alkaloids	+	+	-	-
2	Carbohydrates	-	+	-	-
3	Glycosides	+	-	+	-
4	Saponins	-	-	-	+
5	Protein and amino acids	-	+	+	-
6	Amino acids	-	-	-	-
7	Phenolic compounds	+	+	+	+
8	Flavonoids	+	-	-	+

9	**Terpenoids**	+	-	+	+
10	**Steroids**	+	+	+	+

Quantitative Estimation

The result for quantitative estimation of plant material also justifies the presence of alkaloids, phenolics, flavonoids and terpenoids with maximum yield in methanol.

Fig 1:



Antioxidant activity

In the present investigation, the commonly accepted assays viz DPPH, FRAP, Phospho molybdenum were used for the evaluation of the antioxidant activity of plant extract. The result shows that extracts from the plant can scavenge the radical to a larger extent which correlates with the use of different extracts. It was noted that the antioxidant activity of methanol extract was higher in all experimental methods than those obtained using other solvent extracts.

Table 2:
DPPH radical scavenging assay

S.No	Sample (µl)	Methanol %	Chloroform %	Benzene %	Aqueous %
1	20	32	29	1	3
2	40	38	39	4	5
3	60	41	42	6	6
4	80	49	47	9	11
5	100	59	51	12	15

Table 3:
Ferric reducing antioxidant power (FRAP)

S.No	Sample (µl)	Methanol %	Chloroform %	Benzene %	Aqueous %
1	20	1.754	1.721	1.618	1.603

2	40	1.791	1.749	1.632	1.618
3	60	1.802	1.778	1.681	1.632
4	80	1.847	1.793	1.693	1.7
5	100	1.891	1.807	1.701	1.717

Table 4:
Phosphomolybdenum antioxidant assay

S.No	Sample (µl)	Methanol %	Chloroform %	Benzene %	Aqueous %
1	20	1.672	1.623	1.517	1.592
2	40	1.702	1.667	1.543	1.604
3	60	1.763	1.701	1.572	1.621
4	80	1.801	1.77	1.589	1.638
5	100	1.818	1.793	1.603	1.651

Fig2:

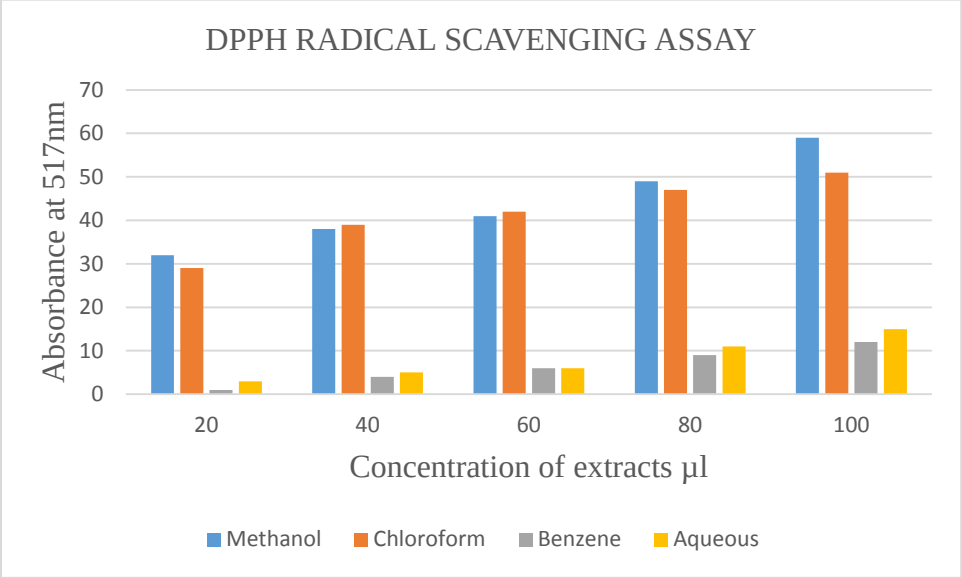
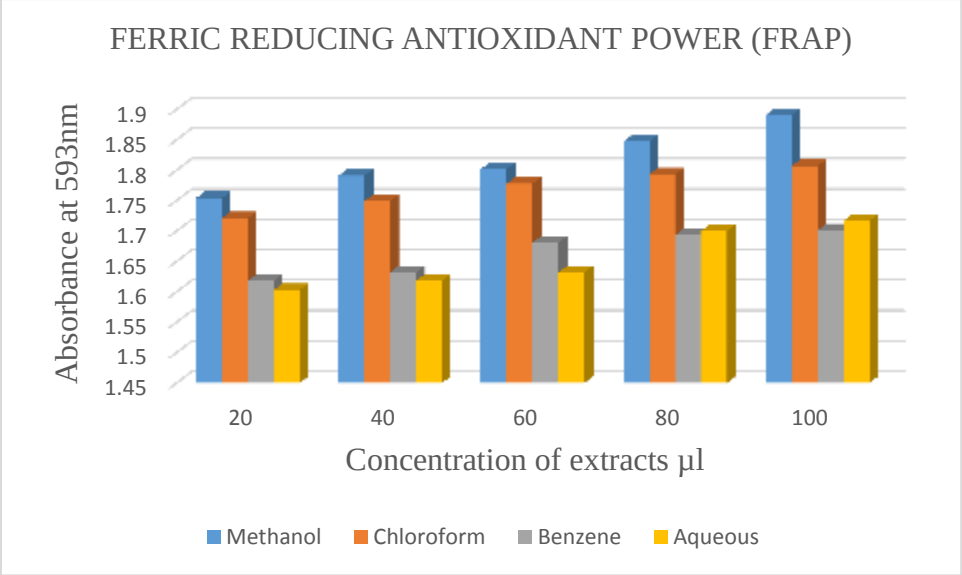


Fig 3:



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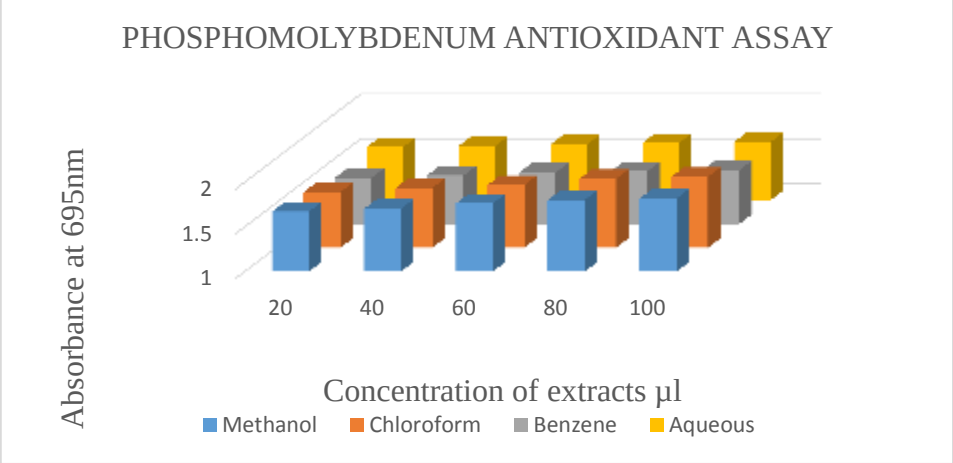


Anti-Inflammatory

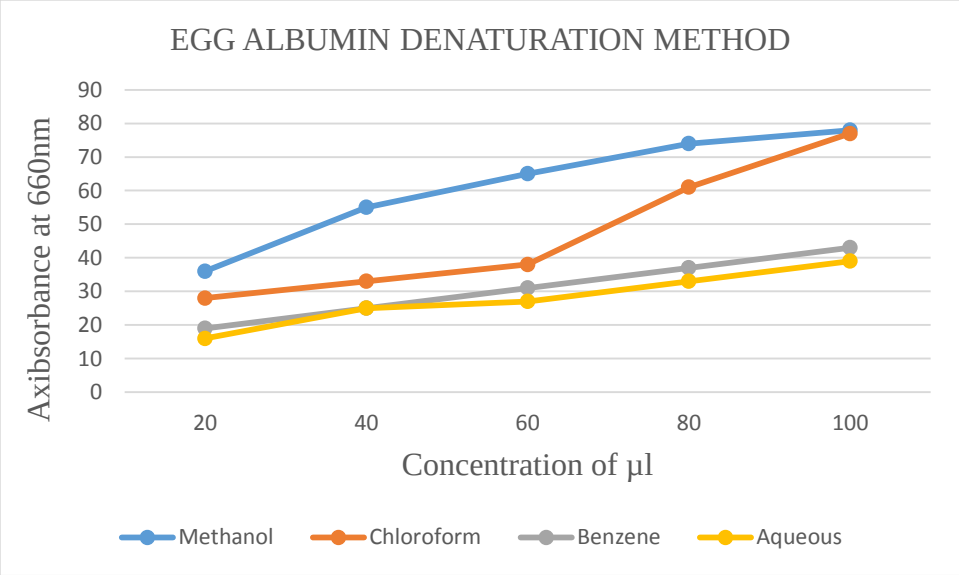
Egg albumin denaturation method

Protein denaturing is a mechanism in which, through the application of external stress or compounds, proteins lose their tertiary structure and secondary structure, such as strong acid or base, concentrated inorganic salt, an organic solvent, or heat. Many biological proteins have been discovered to lose their biological function when denatured, which is a major cause of inflammation. The ability of plant extracts to prevent protein denaturation was investigated as part of an investigation into anti-inflammatory behavior. It was effective in inhibiting albumin denaturation induced by the sun. In chloroform and methanol extracts, maximum inhibition was observed at 77% and 78%. The fractions of the extract act as free radical inhibitors or scavengers.

Table 5:
Inhibition of albumin denaturation

S.No	Sample (µl)	Methanol %	Chloroform %	Benzene %	Aqueous %
1	20	36	28	19	16
2	40	55	33	25	25
3	60	65	38	31	27
4	80	74	61	37	33
5	100	78	77	43	39

Fig 5:



Anti -Diabetic Activity

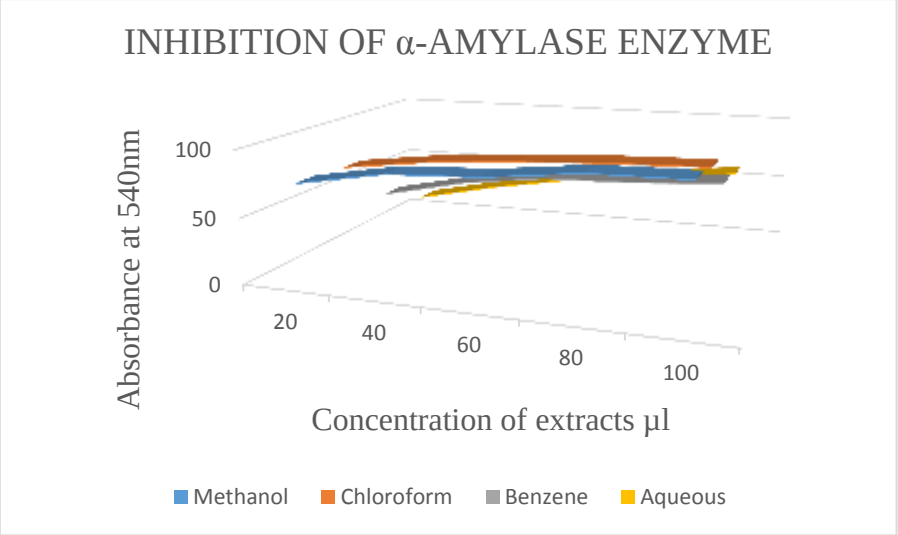
Inhibition of α-amylase enzyme

Plant extracts typically demonstrate successful inhibition of the amylase enzyme, according to studies. α-amylase is an enzyme that hydrolyses broad alpha bonds that bind polysaccharides such as starch and glycogen to produce di-saccharides such as maltose, which will further hydrolyze alpha-glucosidase to produce glucose like mono-saccharides. Alpha-amylasis inhibitors bind to the alpha polysaccharide bond and avoid the mono and di-saccharide polysaccharide degradation. Compared to the normal, all plant extracts display elevated inhibitory activity.

Table 6:
Inhibition of α-amylase enzyme

S.No	Sample (µl)	Methanol %	Chloroform %	Benzene %	Aqueous %
1	20	74	72	35	16
2	40	86	81	50	31
3	60	89	86	59	43
4	80	97	90	61	51
5	100	98	92	66	59

Fig 6:



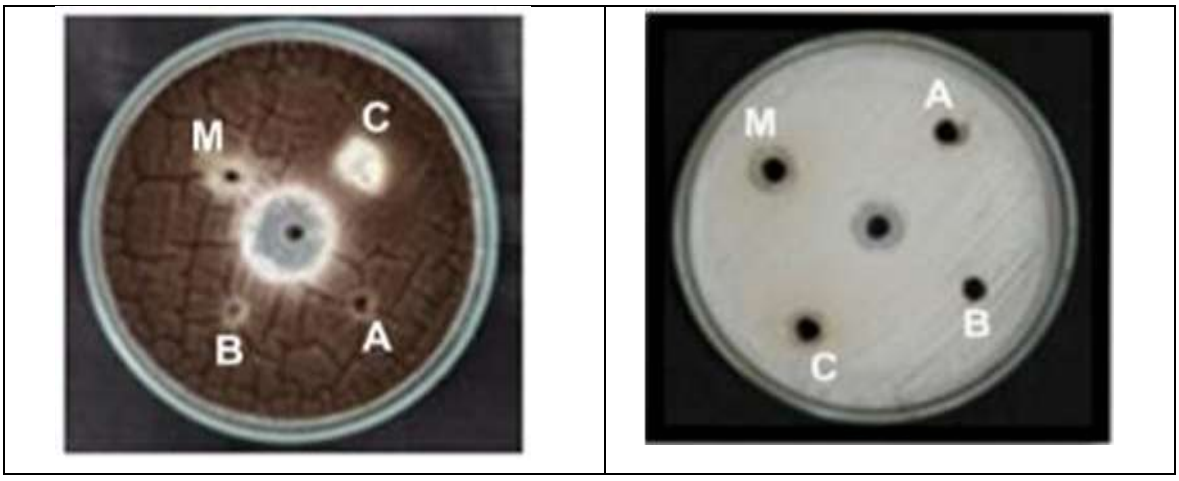
Anti-fungal Activity

In thus study, extracts of methanol and chloroform were observed to have anti-fungal activity against pathogens *Aspergillus niger* and *Candida albicans* except benzene and aqueous extracts.

S.No	Extracts	Aspergillus niger	Candida albicans
		Zone of Inhibition (mm) against different extracts	
1	Methanol	0.7	0.6
2	Chloroform	0.5	0.3
3	Benzene	Nil	1.1
4	Aqueous	Nil	Nil

Fig 7:

Anti-fungal activity of plant extract against human pathogens



Aspergillus nigerCandida albicans

M:Methanol C:Chloroform B:Benzene A:Aqueous

10. Conclusion

The current investigation revealed that due to the existence of phytochemicals, *Tarlmouniaelliptica* (DC.) is a promising potential source of useful medicinal drugs and will be useful in the treatment of various diseases for the formulation of new drugs in pharmaceuticals. Further studies are needed however, to isolate the active principle from the crude drug extract and to understand its mechanism of action concerning protein binding, which will improve the correct, accurate, efficient and safe production of drugs.

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