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Phytochemical Analysis and Application Studies of *Prosopis juliflora* and *Parthenium hysterophorus*

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Abstract: ***Medicinal plants have been used in all cultures as a source of medicine to treat the diseases. Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. Common weed plants were taken for this study such as Prosopis juliflora and Parthenium hysterophorus. Prosopis juliflora and Parthenium hysterophorus plants were collected from the road side of the street at Puzhal, Chennai. Plants showing therapeutic property from screening of the phytochemical constituents which are very helpful to produces and manufacture of new drugs from these phytochemical compounds present in the extracts. Aqueous, Ethanol and Chloroform solvents were used for this study and the extracts were separated into wet and dry sample extracts according to the preparation of extracts method. The extracts of Prosopis juliflora and Parthenium hysterophorus was used against anti bacterial and anti fungal assay to determine the zone of inhibition. Both the plant extracts of Prosopis juliflora and Parthenium hysterophorus exhibit the thrombolytic and anticoagulant properties. The anti inflammatory activity and anti diabetic activity of Prosopis juliflora and Parthenium hysterophorus extracts was observed and studied. The lavicidal activity was done and the results were observed within few seconds the larvae died after addition of extracts to the larvae. All the 12 samples was carried on cytotoxic effect but they has a less effect on the VERO cell lines hence the extracts does not have cytotoxicity effect on cells. The extracts reduce the number of cancer cells which shows it has anticancer property in the extracts.***

1. Introduction

"Let food be your medicine and let medicine be your food." attributed by Hippocrates. Plants are environmental friendly and clean factories. They produce a wealth of novel and biologically active chemicals. Medicinal plants have been used in all cultures as a source of medicine to treat the diseases. The widespread use of herbal remedies and healthcare preparations is described in the Vedas and the Bible. Medicinal Plants have been used for thousands of years to flavor and conserve food, to treat health disorders and to prevent diseases including epidemics. Plants have always been a source of natural product for the treatment of various diseases. About 70– 80% of the world populations, particularly in the developing countries, rely on non-conventional medicine in their primary healthcare as reported by the World Health Organisation. *Prosopis juliflora*, a member of family *Leguminosae*, is found in arid and semi-arid regions of India. It has been used as a folk remedy for catarrh, cold, diarrhea, dysentery, excrescences, flu, hoarseness, inflammation, measles, sore throat and in healing of wounds (Hartwell, 1971). Several alkaloids have been isolated from leaf extracts having pharmacological properties (Ahmad et al., 1988, 1989; Aqeel et al., 1989). *Parthenium hysterophorus* L. from the family of *Asteraceae*, popularly known as Congress weed. It's seeds are ability to germinate in all seasons of the year makes it a constantly flourishing of the vegetation. It also reported as a remedy for against hepatic amoebiasis. South American Indians use the roots to cure ambiotic dysentry, whereas parthenin is a toxin of Parthenium found pharmacologically active against neuralgia and certain types of rheumatism. It is applied externally on skin disorders and a remedy for a wide variety of ailments.

2. Materials and Methods

Collection of Plants: Fresh *Prosopis juliflora* and *Parthenium hysterophorus* were collected directely from the road side of the street at Puzhal, Chennai and washed thoroughly with the tap water to remove the soil and dirt form the leaves and stem. They washed plants are cut into small pieces for plant extraction process.

Materials Required: Small pieces of leaves, stem and flowers freshly from *Prosopis juliflora* and *Parthenium hysterophorus* were taken for the wet sample extraction. Mortar and pestle**.** Soxhlet apparatus and filter paper. 6 glass and plastic bottles, Funnel and conical flask. Solvents - (Distilled water) Aqueous, Ethanol and Chloroform.

Methodology for the Preparation of Plant Extracts Wet and Dry Samples: The Fresh plants samples were properly cleaned to remove the dirt or dust which present on the surface. The Plant samples were cut into small pieces and weighed properly for wet samples. The plant samples were dried under the shade for 10 to 15 days for the dry sample extraction. The Plant samples were powered using mixer and weighed properly. The weighed samples separate into 3parts for further extraction process.10gms of the weighed sample was used for the Aqueous extraction by 100ml Distilled water as solvent using mortar and pestle.10gms of the weighed sample was used for the Ethanol extraction by 100ml Ethanol as solvent using Soxhlet apparatus (24hrs). 10gms of the weighed sample was used for the Chloroform extraction by 100ml Chloroform as solvent using Soxhlet apparatus (24hrs).The samples were extracted and filtered using filter paper and stored in the glass and plastic bottles for the further tests.

Qualitative Phytochemical Screening: Test for Alkaloids, Protein, Flavonoids, Tannins, Terpenoids, Phenols, Carbohydrates, Saponines, Aminoacids, Glycosides,

Steroids was done by standard protocol given in LAKSHMIBAI R, AMIRTHAM D et al., 2015.

Quantitative Analysis: The Total Alkaloids, Protein, Flavonoids, Tannins, Terpenoids, Phenols, Carbohydrates, Saponines, Aminoacids, Glycosides, Steroids content determination was done by standard protocol given in KRWASHNAVENI MARIMUTHU AND DHANALAKSHMI RAVI et. al., 2014.

3. Application Studies

1) Evaluation of *In-Vitro* Antimicrobial Activity

Materials Required: Bacterial culture Anti-bacterial analysis was done using different organisms namely *(Bacillus spp, Pseudomona spp, Staphylococcus aureus* and *Klebsiella spp).* Muller Hinton Agar, Well Borer, Sterile cotton swabs, Ethanol, 200µl and 1000µl tips and pipette, Plant extraction samples, Nutrient Broth/agar. The inoculums is prepared in the Nutrient agar and transferred in to the Nutrient broth as working culture for the further use.

Antibacterial activity: The antibacterial activity of the *Prosopis juliflora* and *Parthenium hysterophorus* was determined by using well diffusion method. Muller Hinton agar was prepared for plating. The standard inoculum suspension was streaked over the surface of the media using sterile cotton swab of the organism and boring the well by using well borer. After that, 100µl the extracts of *Prosopis juliflora* and *Parthenium hysterophorus* samples were poured into the wells. Finally, the inoculated plates were incubated for 24 to 48 hours at 37^{o}C for bacteria. The zone of inhibition was measured and noted.

Antifungal activity: The antifungal activity of extracts was assayed through screening of the *Candida spp and Aspergillus spp* fungi, by the diffusion technique on Sabouraud Dextrose Agar growth medium. The standard inoculum suspension from that loopful was suspended in the 0.8% saline water and this suspension 200µl was streaked over the surface of the media using sterile cotton swab of the organism boring the well by using well borer. After that, 100µl the extracts of *Prosopis juliflora* and *Parthenium hysterophorus* samples were poured into the wells. Finally, the inoculated plates were incubated for 48 to 96 hours at 28^{o}C to 37°C for fungi. The zone of inhibition was measured and noted.

2) In Vitro Antioxidant Activity:- 1, 1 - DIPHENYL – 2 PICRYL HYDRAZYL (DPPH) radical Scavenging

DPPH assay was carried out after making some modifications in the standard protocol. 1.5 ml of 0.1mM DPPH solution was mixed with 1.5ml of various concentrations of plant extract. The mixture was shaken vigorously and incubated at room temperature for 30 minutes in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm by a UV Spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%) = [(A control – A test) / A control] × 100

3) Anti Coagulation Assay: Determination of PT (Prothrombin Time)

The fresh goat blood was collect from the butcher shop and allowed to clot in the outside at room temperature. The clot blood was cut into small pieces and put in the tubes. The 200µl of samples were added to the clot blood tubes and checked for the clot lysis.

4) Thrombolytic Activity

Whole blood was drawn from healthy human volunteers without a history of contraceptives or anticoagulant therapy. Each drop of blood was placed in sterile tile for the total samples and a control.10µl of samples were added to the each drop of blood except control and mixed well. The clotting time is observed and noted.

5) Anti-Inflammatory Activity10% V/V RBC Solution

10ml of packed RBC solution was mixed with equal volume of PBS buffer solution. This was added to 180ml of PBS buffer solution. Finally it contains 200ml of 10% v/v of RBC solution.

Preparation of Plant Extract

1 ml of plant extract was added to 1ml of buffer solution. This 2ml of extract in buffer solution was mixed gently with 2ml of RBC suspension. Previous step was duplicated in 2 sets of tubes. One set was incubated at 54^{o}C for 20 minutes and another one set was incubated at 10^{o}C for 20 minutes. After incubation, the sample was allowed to stand for 30minutes and the haemoglobin content in the supernatant was measured in UV spectrophotometer at 540nm. Percentage inhibition of haemoglobin by the extract was calculated.

$$1 - \frac{(OD_2 - OD_1)}{(OD_3 - OD_1)} \times 100$$

OD_1 - absorbance of test sample unheated, OD_2- absorbance of test sample heated, OD_3 - absorbance of control sample heated.

6) Anti Diabetic Activity by α Amylase Method

0.4 To 2 ml of maltose standard with concentration range from 0.4, 0.8, 1.2, 1.6, 2.0 (800µg to 2000µg) and volume in all test tubes were made up to 5 ml with distilled water.1ml of 3, 5 DNSA reagent was added to all the test tubes and kept in water bath for 10 minutes. The contents were then cooled and 1ml of sodium potassium tartarate was added. The orange red colour was developed which was then measured at 540nm. 1ml of buffer, substrate and NaCl was added to the blank as well as in the labelled unknown tubes and control tubes. Then 0.2 ml of 12 samples was added to the unknown test tubes and incubated in the room temperature for 10 to 15 minutes.2 ml of DNSA was added to arrest the reaction. 0.2 ml of unknown samples was added to the control tubes and incubated all the tubes in the boiling water bath for 10 minutes and then cooled and 1 ml of sodium potassium tartarate was added to both the tubes T and C. The colour developed was read at 540nm.Then the

OD values were plotted in the standard graph (maltose) and then total activity was calculated using formula given below for both the samples.

$$\text{Total activity} = \frac{\mu g/ml \text{ of maltose} \times 1 \times 1000}{\text{molecular weight of maltose} \quad \text{Incubation time}}$$

7) In-Vitro Assay for Anti Cancer and Cytotoxicity Activity (MTT ASSAY) Materials:

Monolayer culture of VERO, BREAST, COLON AND HELA cell lines, Minimal Essential Media (MEM) without FCS, MTT, 96 well plate, 1ml, 2ml pipettes, micropipettes and tips, Discarding jar, Gloves and ethanol.

Methodology

Cell (1×10^5/ well) (VERO, BREAST, COLON, AND HELA CELLS) were plated in 96 well plates and incubated at 37°C with 5% CO_2 condition for 24hrs.After the cell reaches the confluence, media was removed from the wells carefully without disturbing the cells by using PBS buffer and Trypsin. The plant samples (100µl) were added and incubated for 24 hours. After incubation, the sample was removed from the well and washed with phosphate buffered saline (pH-7.4) or MEM without serum.100µl / well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazoium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with ELISA reader using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells/ A570 of control cells × 100

8) In-Vitro Larvicidal Activity

24well plate and Mosquito Larvae (*Culex quinquefasciatus*)

Procedure: 5 larvae (mosquito) per well was added in the test wells.Then 1ml of sample was added to the test wells. The larvae was died in few seconds with extracts.

4. Results and Discussion

Collection of Plants: The fresh *Prosopis juliflora* and *Parthenium hysterophorus* were collected directly from the road side of the street at Puzhal, Chennai. The similar method of plant collection has been reported in a research work done (LAKSHMIBAI.R AND D.AMIRTHAM et al., 2018).

Preparation of Plant Extracts: The cooled extraction were done by motor and pestal and the hot extraction by Soxhlet apparatus of plant samples. The extraction was separated by filter paper to get purified sample. The total wet and dry plant sample extracts were collected and purified and stored for the further application studies.

Qualitative Phytochemical Screening

Table 1: Prosopisjuliflora Wet sample

S.No	Tests	D.Water	Ethanol	Chloroform
1	Alkaloids	+	+	+
2	Proteins	+	-	+
3	Flavonids	+	+	+
4	Tannins	+	+	+
5	Terpenoids	-	-	-
6	Phenols	+	+	+
7	Carbohydrates	-	-	+
8	Saponins	+	-	+
9	Amino acids	-	-	-
10	Glycosides	-	+	+
11	Sterols	-	-	-

Table 2: *Prosopis juliflora* Dry sample

S.No	Tests	D.Water	Ethanol	Chloroform
1	Alkaloids	+	+	+
2	Proteins	+	+	+
3	Flavonids	+	+	+
4	Tannins	+	+	+
5	Terpenoids	-	+	+
6	Phenols	+	+	+
7	Carbohydrates	+	+	+
8	Saponins	+	+	-
9	Amino acids	+	+	+
10	Glycosides	+	-	-
11	Sterols	-	-	-

Table 3: *Parthenium hysterophorus* Wet sample

S.No	Tests	D.Water	Ethanol	Chloroform
1	Alkaloids	+	+	+
2	Proteins	+	+	+
3	Flavonids	+	+	+
4	Tannins	+	+	+
5	Terpenoids	+	+	-
6	Phenols	+	+	+
7	Carbohydrates	+	+	+
8	Saponins	-	-	-
9	Amino acids	-	+	+
10	Glycosides	+	-	-
11	Sterols	+	-	-

Table 4: *Parthenium hysterophorus* Dry sample

S.No	Tests	D.Water	Ethanol	Chloroform
1	Alkaloids	+	+	+
2	Proteins	+	+	+
3	Flavonids	+	+	+
4	Tannins	+	+	+
5	Terpenoids	-	+	+
6	Phenols	+	+	+
7	Carbohydrates	+	+	+
8	Saponins	-	+	-
9	Amino acids	-	+	+
10	Glycosides	+	-	-
11	Sterols	-	-	-

1) **Test for Alkaloids:** A. Dragendroff's test: red precipitate was observed and it indicates the presence of alkaloids in the particular sample. The similar results have been reported for the alkaloids in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015). B. Mayer's test: The white creamy precipitate was observed and it indicates the presence of alkaloids in the

particular sample. The Mayer's reagent was made up of Potassium Mercuric Iodide solution.

2) **Test for Protein:** Biuret's test: blue or violet colour was observed and it indicates the presence of Protein in the sample. The similar results have been reported for the proteins in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
3) **Test for Flavonoids:** Lead acetate test: coloured precipitate was observed and it indicates the presence of flavonoids in the extracts. The similar results have been reported for the flavonoids in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
4) **Test for Tannins:** Ferric chloride test: dirty green was observed and it indicates the presence of tannins. The similar result has been reported for the tanninsin research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
5) **Test for Terpenoids:** reddish brown colour was observed in the lower layer and it indicates the presence of terpenoids in the sample. The similar results has been reported for the terpenoidsin research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
6) **Test for Phenols:** blue, green and black colour was observed and it indicates the presence of Phenols. The similar results have been reported for the phenolsin research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
7) **Test for Carbohydrate:** Fehling's test: red precipitate was observed and it indicates the presence of carbohydrates. The similar results have been reported for the carbohydrates in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015). Molisch's test: The interface was observed for a purple colour indicates the presence of carbohydrate in the sample.
8) **Test for Saponins:** The foam was observed and indicates the presence of saponins. The similar results have been reported for the saponins in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
9) **Test for Aminoacid:** Ninhydrin test: blue colour was observed and indicates the presence of aminoacid in the sample. The similar results have been reported for the aminoacid in research work done (Lakshmibai R, AMIRTHAM D et al., 2015).
10) **Test for Glycosides:** greenish blue colour was observed and it indicates the presence of glycosides in the extract. The similar results have been reported for the glycosides in research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).
11) **Test for Steroids:** reddish violet at the lower layer of test tube was observed and indicates the presence of steroids. The similar results have been reported for the steroidsin research work done (LAKSHMIBAI R, AMIRTHAM D et al., 2015).

The Phytochemical screening of the extracts of *Prosopis juliflora and Parthenium hysterophorus* were done for dry and wet samples. In *Prosopis juliflora* wet sample test for Alkaloid, Protein, Flavonids, Tannins, Phenols, Saponins were found to be positive. Terphenoids, Carbohydrates, Aminoacid and Glycosides and Sterols were found to be negative. In *Prosopis juliflora* dry samples test for Alkaloids, Protein, Flavonids, Tannins, Phenol, Carbohydrates, Saponines, Aminoacids were found to be positive. Terpernoids, Glycosides and Sterols were found to be negative. In *Parthenium hysterophorus* wet samples test for Alkaloids, Protein, Flavonids, Tannins, Terpenoids, Phenol, Carbohydrates, Aminoacid were found to be positive. Glycosides, Sterols, Saponines was found to be negative. In *Parthenium hysterophorus*dry samples test Alkaloids, Protein, Flavonids, Tannins, Phenols, Carbohydrates, Amino acid and Terpenoids was found to be positive. Sterols, Glycosides, Saponines were found to be negative. (LAKSHMIBAI R, AMIRTHAM D et. al., 2015) in their research paper has the similar results we obtained.

Quantitative Analysis

Table 5: Quantitative analysis results for Alkaloids, Proteins, Flavonids and Tannins

Extracts	*Alkaloids*	*Proteins*	*Flavonids*	*Tannins*
W.P.D	5.8±0.2	63±0.75	5.7±1.25	36.3±0.04
W.P.E	9.6±0.33	20±0.4	4±0.8	46.3±0.04
W.P.C	5.4±0.2	6±0.25	9±0.8	13.7±0.08
D.P.D	10.4±0.4	47±0.5	7.33±0.5	58.6±0.07
D.P.E	11±0.2	70±1	6.33±0.5	33.5±0.06
D.P.C	2.8±0.3	3±0.75	3±0.8	8.5±0.06
W.PA.D	9.2±0.33	7±0.7	8.33±0.5	40.2±0.03
W.PA.E	12.6±0.2	33±0.75	7±0.8	55.1±0.02
W.PA.C	1±0.2	2±0.5	5±0.2	2.7±0.08
D.PA.D	9.4±0.2	23±0.75	4±0.5	41.3±0.04
D.PA.E	10.4±0.4	20±0.2	6±0.6	28.6±0.07
D.PA.C	10.6±0.2	2.5±0.3	2±0.2	6.8±0.09

Table 6: Quantitative analysis results for Terpernoids, Phenol, Carbohydrates, Saponines

Extracts	*Terpernoids*	*Phenol*	*Carbohydrates*	*Saponines*
W.P.D	27.8±0.3	92.5±0.2	11.5±0.5	67.5±2.04
W.P.E	8.7±0.8	112±0.3	17.5±0.5	107±1.9
W.P.C	42±0.5	1±0.2	3±0.2	16.83±2.9
D.P.D	89±0.2	18.5±0.6	18.5±0.5	47.5±2.04
D.P.E	36.2±0.3	41±0.4	13±0.2	54±2
D.P.C	55±0.2	1.5±0.5	4±0.2	40±0.4
W.PA.D	60.5±0.6	6±0.6	29±0.2	90±0.5
W.PA.E	26.7±0.3	75.5±0.6	6±0.5	54.5±0.7
W.PA.C	45.6±0.4	106±0.2	2±0.6	6.5±0.7
D.PA.D	78.4±0.6	112±0.5	13±0.8	88±0.5
D.PA.E	36.8±0.2	100±0.3	26±0.6	46.5±0.7
D.PA.C	43±0.4	6.5±0.5	2±0.5	32±0.5

Table 7: Quantitative analysis results for Amino acids, Glycosides, Sterols

Extracts	*Amino Acids*	*Glycosides*	*Sterols*
W.P.D	8.6±0.6	9.17±4.25	1.5±0.2
W.P.E	11.4±0.7	50.5±0.6	15.7±0.7
W.P.C	4±0.5	15±0.7	8.4±0.8
D.P.D	17.2±0.4	27.5±0.6	16.1±0.3
D.P.E	6.6±0.4	40.3±3	16.3±0.04
D.P.C	2.6±0.7	5±0.7	16.2±0.03
W.PA.D	8±0.6	62.5±0.6	12±0.04
W.PA.E	9.4±0.5	27.5±2	16.5±0.6
W.PA.C	3.4±0.7	60±0.7	16.7±0.8
D.PA.D	6.3±0.6	30±0.9	16.2±0.5
D.PA.E	13.4±0.8	47.5±0.3	16.8±0.2
D.PA.C	1.2±0.2	25±0.3	3.8±0.05

The Plant sample extracts contains high amount of Protein, Terpernoids, Phenols and Saponines, Amino acids. Alkaloids, Flavonids, Tannins, Carbohydrates, Glycosides and Sterols are present in moderate amount. The Quantitative analysis values are obtained and represents unit in mg/100ml concentration. From table 5 test for Alkaloids the highest value was 12.6±0.2mg/100ml by W.PA.E and the lowest value was 1±0.2mg/100ml by W.PA.C sample extracts. The test for Proteins the highest value was 63±0.75mg/100ml by W.P.D extract and the lowest value was 2±0.5 mg/100ml by W.PA.C extract. The test for Flavonids the highest value was 9±0.8mg/100ml by W.P.C extract and the lowest value was 2±0.2mg/100ml by D.PA.C extract. The test for Tannins the highest value was 58.6±0.07mg/100ml by D.P.D extract and the lowest value was 2.7±0.08mg/100ml by W.PA.C extract. From table 6 test for Terpernoids the highest value was 89±0.2mg/100ml by D.P.D extract and the lowest value was 8.7±0.8mg/100ml by W.P.E sample extracts. The test for Phenol the highest value was 112±0.5mg/100ml by D.PA.D extract and the lowest value was 1±0.2mg/100ml by W.P.C extract. The test for Carbohydrates the highest value was 29±0.2mg/100ml by W.PA.D extract and the lowest value was 2±0.5mg/100ml by D.PA.C extract. The test for Saponines the highest value was 107±1.9mg/100ml by W.P.E extract and the lowest value was 6.5±0.7mg/100ml by W.PA.C extract. From table 7 test for Aminoacids the highest value was 17.2±0.4mg/100ml by D.P.D extract and the lowest value was 2.6±0.8mg/100ml by D.P.C sample extract. The test for Glycosides the highest value was 62.5±0.6mg/100ml by W.PA.D extract and the lowest value was 5±0.7mg/100ml by D.P.C sample extract. The test for Sterols the highest value was 16.8±0.2mg/100ml by D.PA.E extract and the lowest value was 1.5±0.2mg/100ml by W.P.D sample extract. The similar results has been reported in research work done (KRWASHNAVENI MARIMUTHU AND DHANALAKSHMI RAVI et. al., 2014).

Antibacterial Activity

Gram Postive

Table 8: *Bacillus spp*

Extracts	*Diameter*
W.P.D	-
W.P.E	10mm
W.P.C	2mm
D.P.D	3mm
D.P.E	8mm
D.P.C	13mm
W.PA.D	-
W.PA.E	3mm
W.PA.C	9mm
D.PA.D	-
D.PA.E	14mm
D.PA.C	13mm

Table 9: *Staphylococcus aureus*

Extracts	Diameter
W.P.D	-
W.P.E	4mm
W.P.C	-
D.P.D	-
D.P.E	-
D.P.C	-
W.PA.D	-
W.PA.E	-
W.PA.C	-
D.PA.D	-
D.PA.E	-
D.PA.C	-

Table 8 represents the diameter values of Zone of inhibition of *Bacillus spp* by the extracts. Table 9 represents the diameter values of zone of inhibition of *Staphylococcus aureus* by the extracts. Dry *Parthenium hysterophorus* Ethanol sample gives the maximum zone of inhibition in *Bacillus spp.* Wet *Prosopis juliflora* Ethanol sample only gives the zone of inhibition on *Staphylococcus aureus* other samples have no zone of inhibition on this species.

Gram Negative:

Table 10: *Klebsiella spp*

Extracts	*Diameter*
W.P.D	3mm
W.P.E	8mm
W.P.C	15mm
D.P.D	7mm
D.P.E	10mm
D.P.C	-
W.PA.D	-
W.PA.E	5mm
W.PA.C	20mm
D.PA.D	10mm
D.PA.E	10mm
D.PA.C	10mm

Table 11: *Pseudomona spp*

Extracts	*Diameter*
W.P.D	-
W.P.E	10mm
W.P.C	-
D.P.D	5mm
D.P.E	3mm
D.P.C	2mm
W.PA.D	-
W.PA.E	4mm
W.PA.C	20mm
D.PA.D	20mm
D.PA.E	20mm
D.PA.C	20mm

Table 10 represents the diameter values of Zone of inhibition of *Klebsiella spp* by the extracts. Table 11 represents the diameter values of zone of inhibition of *Pseudomona spp* by the extracts. Wet *Prosopis juliflora* chloroform sample gives the maximum zone of inhibition in *Klebsiella spp.* Dry samples of *Parthenium hysterophorus* gives the maximum zone of inhibition on *Pseudomona spp.* The diameter was around 20mmother samples have moderate zone of inhibition on this species. The similar method was followed and reported in research work done (LAKSHMANAN KRWASHNAVIGNESH, ARUMUGAM et. al., 2013).

Antifungal Activity

Table 12: *Candida spp*

Extracts	*Diameter*
W.P.D	4mm
W.P.E	7mm
W.P.C	9mm
D.P.D	-
D.P.E	5mm
D.P.C	1mm
W.PA.D	7mm
W.PA.E	6mm
W.PA.C	6mm
D.PA.D	-
D.PA.E	9mm
D.PA.C	15mm

Table 13: *Aspergillus spp*

Extracts	*Diameter*
W.P.D	10mm
W.P.E	10mm
W.P.C	-
D.P.D	18mm
D.P.E	17mm
D.P.C	-
W.PA.D	20mm
W.PA.E	3mm
W.PA.C	5mm
D.PA.D	-
D.PA.E	7mm
D.PA.C	20mm

Table 12 represents the diameter values of Zone of inhibition *Candida spp* by the extracts. Table 13 represents the diameter values of zone of inhibition *Aspergillus spp* by the extracts. Dry *Parthenium hysterophorus* chloroform sample gives the maximum zone of inhibition in *Candida spp*. Dry samples of *Parthenium hysterophorus and Prosopis juliflora* gives the maximum zone of inhibition on *Aspergillus spp.* The diameter was around 10 to 20mmother samples have moderate zone of inhibition on this species. The similar method was followed in research work done (LAKSHMANAN KRWASHNAVIGNESH, ARUMUGAM MAHALAKSHMIPRIYA et. al., 2013).The antimicrobial effects of the extracts were evaluated against the pathogens by agar well diffusion method. The extracts inhibit the growth of the bacterial and fungal organisms in the antimicrobial activity. The zone of inhibition was recorded at the concentration100µl/ml. The extracts inhibit the growth of the bacterial and fungal organisms in the antimicrobial activity. The zone of inhibition was recorded at the concentration of 100µl/ml.

Antioxidant Activity

Table 14: Antioxidant DPPH Assay For The Plant Extracts

Topic	*Concentration (µl)*	*Absorbance (517nm)*	*% Inhibition*
Control	Positive	2	-
W.P.D	100	0.03	98.5%
W.P.E	100	0.01	99.5%
W.P.C	100	0.00	100%
D.P.D	100	0.05	97.5%
D.P.E	100	0.00	100%
D.P.C	100	0.02	99%
W.PA.D	100	0.06	97%
W.PA.E	100	0.01	99.5%
W.PA.C	100	0.01	99.5%
D.PA.D	100	0.05	97.5%
D.PA.E	100	0.01	99.5%
D.PA.C	100	0.01	99.5%

The results of DPPH assay for the plant extracts was represent in table 14. The plant extracts shows the greater and better radical scavenging activity in the antioxidant DPPH assay. Antioxdiants are compounds which inhibt the oxidative activity or oxidation and help to increase the life of the matter (CHIRAG et al, .2013).

Thrombolytic Activity: Thrombolytic activity was performed for the plant extracts and the clot lysis was observed positive on only 4 samples. They are Wet *Prosopis juliflora* Ethanol – 30 seconds to lysis the blood clot. Dry *Prosopis juliflora* Ethanol sample took 40 seconds to lysis the blood clot. Wet *Parthenium hysterophorus* Ethanol sample took 45 seconds to lysis the blood clot. Dry *Parthenium hysterophorus* Ethanol sample took 35 seconds to lysis the blood clot. Other sample extracts did not show thrombolytic activity. The similar results have been reported in research work done (HOSSAIN *et al*., 2015).

Anti Coagulation Assay: Determination of PT (Prothrombin Time)

Table 15: Anti coagulation assay results for the extracts

Extracts	Time (sec)
W.P.D	190
W.P.E	10
W.P.C	90
D.P.D	150
D.P.E	10
D.P.C	120
W.PA.D	180
W.PA.E	10
W.PA.C	50
D.PA.D	170
D.PA.E	10
D.PA.C	25
CONTROL	40

Table 15 represents the anticoagulation assay results for the extracts. The extracts act against coagulation in the fresh blood. The clotting time after adding the extracts was observed and recorded. The maximum amount of timing to take clot was 190 seconds and the minimum amount of timing was 10 seconds. Clotting time was the time needed by the sample to coagulate the blood in invitro condition (DAYYAL et al, . 2016).

Anti-Inflammatory Activity

Table 16: Anti inflammatory assay results for the extracts

Extracts	*% Inhibition*
W.P.D	76.5%
W.P.E	80%
W.P.C	26.9%
D.P.D	78%
D.P.E	77%
D.P.C	82%
W.PA.D	90%

W.PA.E	92%
W.PA.C	93%
D.PA.D	95%
D.PA.E	89%
D.PA.C	94%

The table 16 represent the results of inhibition in percentage. The highest value was Dry *Parthenium hysterophorus* Distilled water sample and the percentage was 95%. The lowest value was Wet *Prosopis juliflora* Chloroform sample and the percentage was 26.9%. The similar results have been reported in research work done (PRIYA *et al*., 2005).

Anti Diabetic Activity by α Amylase Method

Table 17: Total activity of Anti diabetic by α Amylase method

Extracts	Total Activity
W.P.D	175.3 IU
W.P.E	175.3 IU
W.P.C	23.4 IU
D.P.D	140.2 IU
D.P.E	373.9 IU
D.P.C	23.4 IU
W.PA.D	23.4 IU
W.PA.E	140.2 IU
W.PA.C	70.1 IU
D.PA.D	444.1 IU
D.PA.E	140.2 IU
D.PA.C	175.3 IU

The table 17 represent the total activity of anti diabetic after adding the extracts to the reagent and incubation period. The highest value was 444.1IU for Dry *Parthenium hysterophorus* Distilled water sample and the lowest value was 23.4 for 3 samples. Other samples have the moderated values. The similar method has been reported in research work done (B DINESHKUMAR, A MITRA et al., 2010).

***In-Vitro* Assay for Anti Cancer and Cytotoxicity Activity (MTT Assay)**

Table 18: %of cell viability in HT 29 cells

Extracts	% of Cell Viability in Colon HT-29 Cancer Cell Lines
W.P.D	69.13%
W.P.E	33.40%
W.P.C	31.03%
D.P.D	60.54%
D.P.E	71.34%
D.P.C	55.56%
W.PA.D	83.34%
W.PA.E	50.30%
W.PA.C	8.15%
D.PA.D	49.58%
D.PA.E	57.77%
D.PA.C	25.49%

Table 19: % of cell viability in HELA cells

Extracts	% of Cell Viability in Hela Cancer Cell Lines
W.P.D	70.22%
W.P.E	53.23%
W.P.C	49.86%
D.P.D	31.18%
D.P.E	92.78%
D.P.C	90.65%
W.PA.D	69.33%
W.PA.E	71.73%
W.PA.C	13.89%
D.PA.D	31.49%
D.PA.E	74.69%
D.PA.C	22.63%

Table 20: % of cell viability in MCF 7 cells

Extracts	% of Cell Viability in Breast MCF 7 Cancer Cell Lines
W.P.D	55.06%
W.P.E	84.37%
W.P.C	59.77%
D.P.D	34.46%
D.P.E	92%
D.P.C	77.62%
W.PA.D	91%
W.PA.E	83.75%
W.PA.C	25.84%
D.PA.D	95%
D.PA.E	91.92%
D.PA.C	27.97%

Table 21: % of cell viability in VERO cells

Extracts	% of Cell Viability in Vero Cancer Cell Lines
W.P.D	91%
W.P.E	89%
W.P.C	71%
D.P.D	88%
D.P.E	92%
D.P.C	77%
W.PA.D	91%
W.PA.E	83%
W.PA.C	84%
D.PA.D	95%
D.PA.E	91%
D.PA.C	57%

Anticancer activity: Table 18, 19 and 20 represents the percentage values of cell viability of certain cancer cell lines against the extracts. The anticancer activity of all the plant sample extracts was done and the results were showed their obtained value of the viable cells. The reduction of viable cells after adding of samples indicates the presence of anticancer property of the sample extracts. The similar results have been reported in research work done (SHASHANK KUMAR, GOUSIA CHASHOO et. al., 2013).

Cytotoxicity activity: Table 21 represents the percentage values of the cell viability of VERO cell lines against the extracts. The percentage of the cell viability was near 100% which means after adding of sample extracts the cells was more tend to proliferating on their own. The proliferation of the cells gives the higher values in percentage. The similar method has been reported in research work done (P SKEHAN, R STORENG, D SCUDIERO et al., 1990).

***In-Vitro* Larvicidal Activity**: In 24 well plate the samples extracts were added to the mosquito larva (*Culex quinquefasciatus*). In few seconds the larvae died in the extracts. The similar method has been reported in research work done (MS KUMAR, S MANEEMEGALAI et al., 2008).

5. Conclusion

Prosopis juliflora and *Parthenium hysterophorus* was subjected to extraction with 3 different solvents which was Aqueous, Ethanol and Chloroform. The Phytochemical screening of the extracts of *Prosopis juliflora* and *Parthenium hysterophorus* revealed the presence of various compounds such as alkaloids, proteins, tannins, amino acids, phenols, flavonoids, steroids, terpenoids, glycosides and saponins. The extracts of *Prosopis juliflora* and *Parthenium hysterophorus* exhibited the antibacterial activity against the 2 Gram positive and 2 Gram negative organisms (*Bacillus spp, Pseudomona spp, Staphylococcus aureus* and *Klebsiella spp)* and zone of inhibition was measured and reported. It was also exhibited the antifungal activity against 2 organisms (*Candida spp and Aspergillus spp)* and zone of inhibition was observed and reported. The anti oxidant activity was carried out by DPPH assay both extracts show near 100% inhibition for the test. The plant extracts shows the greater and better radical scavenging activity in the antioxidant DPPH assay. The *Prosopis juliflora* and *Parthenium hysterophorus* extracts were exhibited in thrombolytic activity to check the time observed to lysis the blood clot by extracts. The *Prosopis juliflora* and *Parthenium hysterophorus* extracts were exhibited in the anti coagulate activity so the Prothrombin Time was calculated and reported. The anti inflammatory activity of *Prosopis juliflora* and *Parthenium hysterophorus* extracts was studied and the both extracts show better inhibition and they were observed. The anti diabetic activity of the *Prosopis juliflora* and *Parthenium hysterophorus* extracts was done by α amylase method and the total activity was observed and reported. All the 12 samples was carried on cytotoxic effect but they has a less effect on the VERO cell lines hence the extracts does not have cytotoxicity effect on cells. The extracts reduce the number of cancer cells which shows it has anticancer property in the extracts. The lavicidal activity was done and the results were observed within few seconds the larvae died after addition of extracts to the larvae. For the future outcome *Prosopis juliflora* and *Parthenium hysterophorus* plants can be micropropagated using invitro condition to obtained a pure hybrid variety of toxic less weed to protect the crops in the field. *Parthenium hysterophorus* plant extract have a toxicity in high amount which can be reduced and use in various field such as cancer therapy, pharmaceutical company etc. *Prosopis juliflora* and *Parthenium hysterophorus* genes can be isolate and modified to incorporated into the crop plants to get resistance against Biotic and Abiotic stress in field.

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