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In vitro and inhibitory activity of pathogens on leaves of *Argemone mexicana* L. and *Premna tomentosa* L.

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Abstract
The leaves of potential medicinal plants such as *A. mexicana* and *P. tomentosa* were. subjected for Phytochemical screening, evaluation of antioxidant activity and antimicrobial studies. In the present study, all the extracts clearly showed the presence of certain important secondary metabolites. High levels of polyphenols and flavonoids were found in ethyl acetate and ethanol extracts, respectively in *P. tomentosa* when compare to the *A. maxicana*. The antioxidant activity of the plant extracts were determined by using DPPH scavenging and reducing power assay. The results clearly indicated that ethyl acetate extract showed better DPPH scavenging activity, in *P. tomentosa* at concentration of 1000 mg/ml of extract. The antimicrobial activity was done by agar well diffusion method against eight bacterial strains viz., The ethanol extract of *A. mexicana* exhibited highest antimicrobial activity against *B. subtilis* when compared with synthetic streptomycin.

Keywords: Phytoconstituents, antioxidant activity, antimicrobial activity, *Argemone mexicana* and *Premna tomentosa*

1. Introduction
Natural products could be potential drugs for humans or live stock species and also these products and their analogues can act as intermediates for synthesis of useful drugs [1]. Plants possess many phytochemicals with various bio activities including antioxidant, anti-inflammatory and anticancer [2]. Secondary metabolites from plants have important biological and pharmacological activities, such as anti-oxidative, anti-allergic, antibiotic, hypoglycemic and anti-carcinogenic [3, 4, 5]. More than 4000 phenolic compounds (flavonoids, monophenols and polyphenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, narigan, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents. Most flavonoids function in the human body as antioxidants.
Antioxidants are vital substance which possesses the ability to protect the body from damage caused by free radical induced oxidative stress. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging from or promoting their decomposition and suppressing such disorders [6]. Plants have been an important source of medicine for thousands of years. Even today, the world health organization estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicines [7]. Medicinal plants constitute one of the main sources of new pharmaceutical and health care products. A whole range of plant-derived dietary supplements, phytochemical and pro-vitamins that assist in maintaining good health and combating disease are now being described as functional ingredients and nutraceuticals. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents, usually associated to a wide range of amphipathic molecules, broadly termed polyphenolic compounds [8].

1.1 Objectives of this studies
1. To extract the phytochemical components from the leaves of *A. mexicana* and *P. tomentosa* using aqueous and solvent extracts.
2. To determine the levels of important phytochemical constituents such as total phenols and flavonoid in the extract of selected medicinal plants.
3. To evaluate the antioxidant activity of crude extracts using DPPH and reducing power assay.
4. To find out antimicrobial potential of concentrated crude extracts against clinically important microbial pathogens.

3. Materials and Methods

3.1 Collection of plant samples

Fresh plants leaves of *Argemone mexicana* L. and *Premna tomentosa* L. were collected from the natural strands of Coimbatore and Namakkal district, Tamil Nadu. The botanical identity of the collected specimens was confirmed by Dr. Gopalan, (Scientist (Rtd.), Department of Botany, Karpagam University, Coimbatore. The voucher specimens are deposited at the Department of Botany, Government Arts College, Coimbatore – 18. Tamil Nadu, India. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

2.2 Crude extract preparation

2.2.1 Solvent extraction

50 g of air- dried powder was taken with 200 ml of Ethyl acetate and Ethanol in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days, the extract was filtered using Whatman No.1 filter paper and the solvent was evaporated to make the final volume to one fourth of the original volume and stored at 40 C in airtight bottles.

2.2.2 Aqueous extraction

50 g of air-dried powder was taken with 200 ml of water in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days, the extract was filtered using Whatman No.1 filter paper and the solvent was evaporated to make the final volume to one fourth of the original volume and stored at 40 C in airtight bottles.

2.3 Preliminary phytochemical screening

Phytochemical screening of Ethyl acetate, Ethanol and Aqueous extracts of *Argemone mexicana* and *Premna tomentosa* were carried out by using standard methods adopted by [9, 10].

2.4 Antioxidant assay

The antioxidant activity of crude extracts was determined by two different *in-vitro* methods such as, DPPH free radical scavenging activity and reducing power assay methods.

2.5 DPPH Radical scavenging activity

DPPH scavenging activity was carried out by the method of Blois, [11]. Different concentrations (1000, 500, 250, 125, and 62.5 mg/ml) of concentrated crude extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1mM ethanol solution of DPPH (1, 1, Diphenyl- 2- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 370 C for 20 min. The control was prepared without plant extracts. Ethanol was used for baseline corrections. The absorbance (OD) of sample was measured at 517 nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

2.6 Reducing power assay

Reducing activity was carried out by using the method of Oyaizu [12]. Different concentrations (1000, 500, 250, 125, and 62.5 mg/ ml) of concentrated crude extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in the test tubes in triplicates. To the test tubes 2.5ml of sodium phosphate buffer and 2.5ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 500C for 20min. After incubation, 2.5ml of 10% TCA was added and were kept for centrifugation at 3000rpm for 10min. After centrifugation 5ml of supernatant were taken and to this 5ml of distilled water was added. To this about 1ml of 1% ferric chloride was added and was incubated at 350C for 20min. The OD (absorbance) was taken at 700nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. The reducing power of the extract is linearly proportional to the concentration of the sample.

2.7 Quantitative Estimation of Total Phenols and Flavonoids

2.7.1 Total phenolic content

Total phenolic contents were determined by Folin Ciocalteu reagent [13]. A dilute extract of each plant extract (0.5ml of 1:10g ml-1) or garlic acid (standard phenolic compound) was mixed with 5ml of Folin Ciocalteu reagent (1:10 diluted with distilled water) and 4ml of 1 M aqueous sodium carbonate. The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 50, 100, 150, 200, 250 mg/ml solution of gallic acid in ethanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/ g-1 of dry mass), which is a common reference compound.

2.7.2 Total flavonoids content

Aluminum chloride colorimetric method was used for flavonoids determination [14]. Each plant extracts (0.5ml of 1:10g/ml) were separately mixed with 1.5ml of ethanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing qucertin solution at concentrations 12.5 to 100g ml^{-1} in ethanol.

2.8 Antimicrobial studies

The antibacterial activity of the crude extracts of *A. mexicana* and *P. tomentosa* was determined by agar-well diffusion method. The isolated bacterial were first cultured in nutrient broth for 24 hrs before use. 200 µl of the standardized cell suspension were spread on a Muller-Hinton agar. Sterile 4 mm diameter of cork-borer was used to bored wells into the MH agar. Approximately 100 µl of 10 mg of each plant extracts were introduced in the wells separately, allowed to stand at room temperature for about 2hrs and then incubated at 37^0C. The plates were observed for zone of inhibition after 24 hrs and compared with streptomycin at a concentration of 10 mg/ml [15].

3. Results

3.1 Phytochemical screening

Ethyl acetate, Ethanol and Aqueous extracts of the selected samples were subjected to qualitative organic analysis (Table-1&2).

3.2 Antioxidant activity

3.2.1 DPPH Scavenging activity

The extracts of all the tested plant specimens possessed free radical scavenging properties, but to varying degrees, ranging

from 9.0 to 76.6% using the organic solvent extraction, generally Ethyl acetate showed better DPPH scavenging activity. A maximum scavenging activity was offered by Ethyl acetate of *P.tomentosa* (76%), *A.mexicana* (70.1 %) followed by ethanol extract of *P.tomentosa* (71 %), *A.mexicana* (67%) and water extract of *P.tomentosa* (67.5 %) and *A.mexicana* (63%) at 1000 mg/ml concetration. (Table-3 & 4).

3.3 Reducing power assay

The reducing power assay of two different plant samples were given in table (5 and 6) which was found to be ranged from 0.067 to 0.8 %. The reducing power of the crude extracts increased in a concentration dependent manner. Among the two different sp, the ethyl acetate extracts of *P.tomentosa* **(**0.8) have exhibited the highest rate of reduction of ferric ions at 1000 mg/ml. When compared with standard ascorbic acid, both the samples of the present study exhibited higher reducing power is all the three crude extracts at 1000 mg/ml concentration.

3.4 Quantitative estimation Polyphenols and Flavonoids

Two different plant tissues using water and two different organic solvents were prepared to quantify the phenols and flavonoids. The concentration of phenols in the examined plant extracts using the Folin-Ciocalteu reagent was expressed in terms of gallic acid equivalent (Table 7). The concentrations of phenols in the examined crude extracts ranged from 364 to 543 mg/g. The highest concentration of phenols was measured in ethyl acetate extracts of *P. tomentosa*. The extracts obtained using more polar solvents had higher concentrations of phenols while the extracts obtained using low polar solvents contained small concentrations. The concentration of flavonoids in two different crude organic and aqueous extracts were determined using spectrophotometric method with aluminium chloride. The results of levels of flavonoids in the tested extracts is shown in (Table 8). The concentration of flavonoids in crude extracts ranged from 109 to 187 mg/g dry sample. High concentrations of flavonoids were present in ethanol extracts of *P.tomentosa.* The concentration of flavonoids in the extracts depends on the polarity of solvents and the type of plant material used for the extraction.

3.5 Antimicrobial activity

Results obtained in the present study revealed that the tested two medicinal plant crude extracts posses potential antimicrobial activity against eight clinically important microbial pathogens (Table 9 and 10) (Plate 1 to 3). The antimicrobial activity was carried out by well diffusion method, the ethanol extracts of *A. mexicana* showed significant activity against *Bacillus* sp and *Sheigella* sp. whereas the ethanol extract of *p. tomentosa* exhibited significance activity *Sheigella* sp. Among the various microorganisms tested for antimicrobial activity against crude extracts of both the samples of the present study, the ethanol extract of *A. mexicana* exhibited highest antimicrobial activity against *B. substils* (19 mm zone of inhibition) when compared with synthetic streptomycin. Leaf extracts of *P.tomentosa* exhibit highest antimicrobial activity against *Shigella* sp (15mm) when as compared with streptomycin.

Tables

Table 1: Preliminary phytochemical screening of Ethyl acetate, Ethanol and aqueous extract of *A. mexicana*.

Phytoconstituents	Plant Extracts		
	Ethyl acetate	Ethanol	Aqueous
Alkaloids	+	+	-
Flavonoids	-	-	+
Saponins	+		-
Steroids	+	+	-
Tannins	+	-	-
Terpenoids	-	+	-

(+) = Indicates Positive (-) = Indicates Negative

Table 2: Preliminary phytochemical screening of Ethyl acetate, Ethanol and aqueous extracts of *P. tomentosa*.

Phytoconstituents	Plant Extracts		
	Ethyl acetate	Ethanol	Aqueous
Alkaloids	+	-	-
Flavonoids	-	+	-
Saponins	+	-	-
Steroids	-	+	-
Tannins	-	+	-
Terpenoids	-	+	-

(+) = Indicates Positive (-) = Indicates Negative

Table 3: DPPH Assay of ethyl acetate, ethanol and aqueous extracts of *A. mexicana**

Concentrations (mg/ml)	Antioxidant activity (%) Plant extracts			Standard (Ascorbic acid)
	Ethyl acetate	Ethanol	Aqueous	
1000	70.1 ± 1.96	67.16 ± 2.75	63.33 ± 1.52	65.34 ± 1.54
500	53.6 ± 1.52	51.6 ± 2.51	42.6 ± 2.08	
250	28.0 ± 2.0	30.3 ± 1.15	26.6 ± 1.5	
125	18.31 ± 2.8	19.3 ± 1.25	12.0 ± 2.09	
62.5	10.0 ± 1.05	11.39 ± 1.54	9.0 ± 1.05	

*Data are mean of three replicates, ± Standard Error

Table 4: DPPH Assay of ethyl acetate, ethanol and aqueous extracts of *P.tomentosa**

Concentrations (mg/ml)	Antioxidant activity (%) Plant extracts			Standard (Ascorbic acid)
	Ethyl acetate	Ethanol	Aqueous	
1000	76.6 ± 2.08	71 ± 1.73	67.5 ± 1.32	65.34 ± 1.54
500	51.3 ± 1.52	56.6 ± 1.87	50.6 ± 1.15	
250	25.3 ± 0.57	31 ± 1.73	29.1 ± 2.08	
125	12.16 ± 1.04	21.9 ± 0.90	19.3 ± 1.52	
62.5	10.8 ± 1.04	11.6 ± 0.55	10.96 ± 1.00	

*Data are mean of three replicates, ± Standard Error.

Table 5: Reducing power Assay of ethyl acetate, ethanol, and aqueous extracts of *A.mexicana**

Concentrations (mg/ml)	Antioxidant activity (%)			Standard (Ascorbic acid)
	Plant extracts			
	Ethyl acetate	Ethanol	Aqueous	
1000	0.793 ± 4.35	0.752 ± 2.51	0.682 ± 2.51	0.685 ± 0.23
500	0.361 ± 1.15	0.519 ± 2.08	0.341 ± 3.60	
250	0.181 ± 1.52	0.341 ± 2.30	0.170 ± 3.0	
125	0.121 ± 0.01	0.211 ± 0.01	0.109 ± 0.02	
62.5	0.084 ± 0.01	0.109 ± 0.01	0.091 ± 0.02	

*Data are mean of three replicates, ± Standard Error.

Table: 6: Reducing power Assay of ethyl acetate ethanol, and aqueous of extracts of *P. tomentosa**

Concentrations (mg/ml)	Antioxidant activity (%)			Standard (Ascorbic acid)
	Plant extracts			
	Ethyl acetate	Ethanol	Aqueous	
1000	0.800 ± 0.03	0.782 ± 0.02	0.715 ± 0.05	0.685 ± 0.23
500	0.441 ± 1.52	0.372 ± 3.21	0.354 ± 2.08	
250	0.261 ± 2.08	0.196 ± 1.52	0.212 ± 2.51	
125	0.123 ± 2.64	0.116 ± 1.5	0.114 ± 0.57	
62.5	0.089 ± 2.64	0.086 ± 1.73	0.067 ± 0.577	

*Data are mean of three replicates, ± Standard Error.

Table 7: Total phenol contents of crude extracts of *A. mexicana* and *P. tomentosa* (mg/GAE 100 g of dry samples)*

Plant Name	Plant extracts		
	Ethyl acetate	Ethanol	Aqueous
A. mexicana	445 ± 0.07	480 ± 1.04	364 ± 0.13
P. tomentosa	543 ± 1.38	468 ± 1.43	369 ± 2.01

*Data are mean of three replicates, ± Standard Error.

Table: 8: Flavonoids contents crude extracts of *A. mexicana* and *P. tomentosa* (mg/QE 100 g of dry samples)*

Plant Name	Plant extracts		
	Ethyl acetate	Ethanol	Aqueous
A. mexicana	165 ± 0.05	184 ± 0.04	181 ± 0.05
P. tomentosa	176 ± 0.43	187 ± 1.12	109 ± 0.31

*Data are mean of three replicates, ± Standard Error.

Table 9: Antimicrobial activity of crude extracts of *A. mexicana*.

Micro organisms	Zone of inhibition (mm)			
	Ethyl acetate	Ethanol	Aqueous	Streptomycin
Staphylococcus aureus	<10	15	-	10
Streptococcus pyogens	-	-	-	-
Bacillus subtilis	<10	19	-	14
Eschericha coli	<10	11	-	-
Klebsiella pneumonia	-	-	-	-
Proteus vulgaris	-	-	-	-
Salmonella typhi	7	7	-	9
Sheigella flexeri	<10	14	-	11

Table 10: Antimicrobial activity of crude extracts of *P. tomentosa*.

Micro organisms	Zone of inhibition (mm)			
	Ethyl acetate	Ethanol	Aqueous	Streptomycin
Staphylococcus aureus	<10	<10	<10	<10
Streptococcus pyogens	<10	<10	-	-
Bacillus subtilis	-	-	-	-
Escherichia coli	-	<10	-	-
Klebsiella pneumonia	-	-	-	-
Proteus vulgaris	-	<10	-	-
Salmonella typhi	5	7	-	9
Sheigella flexeri	<10	15	-	12

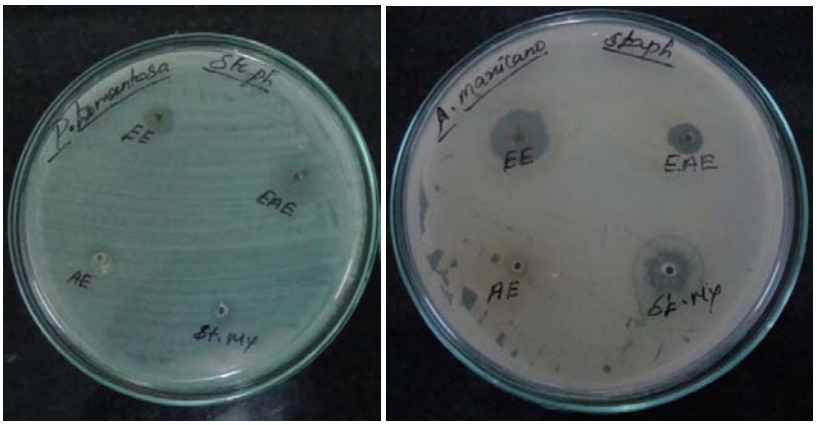
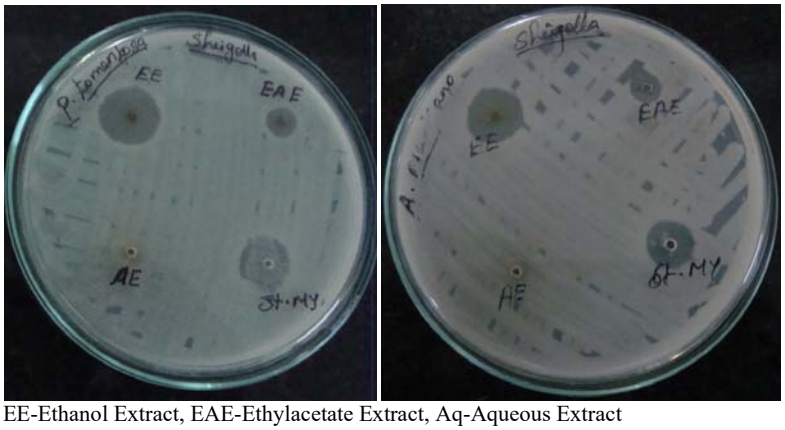


Plate 1: Antibacterial activity of crude extracts of *A. mexicana* and *P. tomentosa* against *Staphylococus aureus*

EE-Ethanol Extract, EAE-Ethylacetate Extract, Aq-Aqueous Extract

Plate 2: Antibacterial activity of crude extracts of *A. mexicana* and *P. tomentosa* against *Bacillus subtilis*



EE-Ethanol Extract, EAE-Ethylacetate Extract, Aq-Aqueous Extract

Plate 3: Antibacterial activity of crude extracts of *A. mexicana* and *P. tomentosa* against *Sheigella dysentry*

4. Discussion

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many diseases, central nervous system injury, gastritis, cancer and AIDS [16]. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in prevention of the free radical formation by scavenging or by effecting promotion of their decomposition and suppression of such disorders [17]. There is growing interest towards natural antioxidants from herbal sources.

Plant materials containing phenolic constituents are increasingly of interest for probing as they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of the antioxidant constituents of plant material in the maintenance of health and protection from coronary heart disease and cancer has been well recognized [18]. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process [19]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [20].

4.1 Phytochemical screening

All plant parts synthesize some chemicals by themselves, to perform their physiological activities. In our present study, the investigated plants have exhibited different kinds of secondary metabolites. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include, alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement, and body building [21].

Alkaloids have been well investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic [22], and anti-inflammatory properties, but there are only few reports about their antimicrobial properties.

Saponins are glycosides occurring widely in plants. They are abundant in many foods consumed by animals and man. Saponin is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolemia, hyperglycemia, antioxidant, anti-cancer, anti-inflammatory [23], central nervous system activities [24], and weight loss etc. Plant steroids are known to be important for their cardiotonic activities, possession of insecticidal, anti-inflammatory [25], analgesic properties [26], central nervous system activities and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics. Out of the six plants, studied steroids are present in *Meliah azadiarch* and *Calotropis gigantea* [24].

Tannins were reported to exhibit antidiabetic [22], anti-inflammatory [27], antibacterial and antitumor activities. It has also been reported that certain tannins were able to inhibit HIV replication selectively besides use as diuretics. Plant tannins have been widely recognized for their pharmacological properties and are known to make trees and shrubs a different meal for many caterpillars [28].

Glycosides were reported to exhibit anti-diabetic characteristics [22]. Cardiac glycosides on the other hand are known to hamper the Na + / K + pump. This results in an increase in the level of sodium ions in the myocytes which then enhance the level of calcium ions. This consequently increases the amount of Ca 2+ ions available for contraction of the heart muscle, which improves cardiac output and reduces distention of heart and thus are used in the treatment of congestive heart failure and cardiac arrhythmia.

4.2 Antioxidant activity

4.2.1 DPPH Free Radical Scavenging Activity

DPPH a stable free radical, has been used to evaluate the antioxidant activity of natural products by measuring the radical quenching capacity in a relatively short period of time. It has been shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and standards to certain extent and hence are said to be strongly dependant on the extract concentration.

The free radical inhibition activity of crude extracts of two different plant specimens were ranged between 9.0 and 76.6 %, which is in agreement with that of the previous reports on *Stachys lavandulifolia* (9.99%), *Ocimum basilicum* (44%) [29].

4.2.2 Reducing power assay

Reducing power assay is often to evaluate the ability of natural antioxidant to donate electron or hydrogen [30]. Samples with high reducing power were reported to have a better ability to electrons. It has been widely accepted that the higher level of absorbance at 700 nm indicates greater reducing power of the test samples [31].

4.3 Polyphenols and flavonoids

The high phenol and flavonoids contents of Peels and tissue of *C. sinesis* may cause high antioxidant activity of this plant. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [32]. As reported, antioxidant activity of fruits and vegetables significantly increases with the presence of high concentration of total polyphenolic content.

4.4 Antimicrobial activity

Many medicinal plants are considered to be the store houses of potential antimicrobial crude drugs as well as sources for novel compounds with antimicrobial activity, with possibly new modes of action. This expectation that some naturally occurring plant compounds can kill antibiotic-resistant strains of bacteria such as *Bacillus cereus*, *Escherichia coli*, *Micrococcus luteus* and *Staphylococcus aureus* has been confirmed [33]. In the past few decades, the search for new anti-infection agents has engaged many research groups in the field of ethnopharmacology. A Pubmed search for the antimicrobial activity of medicinal plants has produced 115 articles during the period between 1966 and 1994. However, in the following decade between 1995 and 2004, this number has more than doubled to 307. In these studies one could find a wide range of criteria related to the discovery of antimicrobial compounds in plants.

In results of our study, on the basis of inhibition zone diameters varied according to microorganisms, but globally, the highest inhibition zone diameters have been recorded with Gram-positive bacteria. It is reported that Gram-positive bacteria tend to be more susceptible since they have only an outer peptidoglycan layer, which is not an effective barrier [34]. *Premna integrifolia* contains important chemical constituents. It was revealed that the leaf extract contains reducing sugar, tannins, flavonoids, steroids, alkaloids, and glycosides which have potential role in its Analgesic and Antimicrobial activity [35].

The antibiotic potential of the extracts of the leaf and stem of *A. mexicana* has been determined against the water borne bacteria. For the comparison of the plant extracts activity control (different types of antibiotic disc) and negative control (only solvent absorbing disc) was used. The negative control showed no activity against all tested bacteria. The positive control showed significant antibacterial activity against water borne bacteria [36].

5. Conclusion

The results of the present study, clearly explained that among the two plant material, the *P. tomentosa* was found to be more potential than the *A. mexicana*. Thus, after conducting certain advance studies, the phytochemical compound may be separated and biosynthesis and structural elucidations can be carried out in future for the effective utilization of this potential medicinal plants.

6. Reference

1. Makkar HPS, Norvsambuu T, Lkhavatsere S, Becker K. Plant secondary metabolites in some medicinal plants of Mongolia used for enhancing animal health and production. Tropicultura. 2009; 27(3):159-167.

2. Wu JY, Yang BB. Anticancer activity of Hemsyaamabitis extract. Life sciences. 2002; 71:2161-2170.
3. Borneo R, Leon EA, Aguirre A, Ribotta P, Cantero JJ. Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their *in vitro* testing in model food system. Food Chem. 2008; 112:664-670.
4. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 2004; 94:550-557.
5. Mulabagal V, Tsay H. Plant Cell Cultures - an alternative and efficient source for the production of biologically Important secondary metabolites. Int. J Appl Sci Eng Tech. 2004; 4:29-48.
6. Akanksha Mishra, Bapat Mrinal M, Tilak Jai C. Devasagayam, and Thomas P.A. Antioxidant activity of *Garciniaindica* and its syrup. Cureent sci. 2006; 91(1):90-93.
7. Tripathi L, Tripathi NJ. Role of biotechnology in medicinal plants. Trop. J Pharm Res. 2003; 2(2):243-253.
8. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacol. 2005; 97(1-2):145-150.
9. Trease GE, Evans WC. Pharmacognosy 11th Edn. Brailliar Tridel and Macmillian Publishers, London. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. J Phytopharacology. 1989; 12(2):224-233.
10. Harborne JB. Phytochemical Methods, A Guide to modern Techniques of Plant Analysis. Chapman and Hall, London. 1973, 33-41.
11. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199-1200.
12. Oyaizu M. Studies on products of browning reaction: Antioxidant activities of products of browning reaction Reid, K.A., Ager, A.K. J, Light, M.E. Mulholland D.A., J. Van Staden. 20 in 05. Phytochemical prepared from glucosamine. Jap. J Nut. 1986; 44:307-15.
13. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001; 73:73-84.
14. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002; 10:178-182.
15. Nagaprashanthi CH, Aleemudd MA, Kannan M. investigation of invitro anthelmintic activity of aqueous leaf extract of tinospora cordifolia international journal of pharmacy and pharmaceutical sciences. 2012; 4(2):673-675.
16. Pourmorad F, Hosseinimehr SJ, Shahabimajd N, Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J Biotech. 2006; 5(11):1142-1145.
17. Souri E, Amin G, Farsam H, Barazandeh Tehrani M. Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. DARU. 2008; 16:283-287.
18. Khalil MY, Moustafa AA, Naguib NY. Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. World J Agri Sci. 2007; 3(4):451-457.
19. Kessler RC, Barker PR, Colpe LJ, Epstein JF, Gfroerer JC, Hiripi E *et al.* Screening for serious mental illness in the general population archives of general psychiatry. 2003, 60(2).
20. Shahidi F, Wanasundara PKJPD. Phenolic antioxidants. Crit. Rev. Food Sci Nut. 1992; 32:67-103.
21. Kubmarawa D, Khan ME, Punah AM. Hassan Phytochemical screening and antibacterial activity of extracts from *Parkia clappertoniana* key against human pathogenic bacteria. J Med Pl Res. 2008; 2(12):352-355.
22. Cherian S, Augusti KT. Insulin sparing action of leucopelargonidin derivative isolated from *Ficus benghalensis* Linn. Indian J Exp Biol. 1995; 33:608- 611.
23. Manach C, Regerat F, Texier O. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. Nutr. Res. 1996; 16:517-544.
24. Argal A, Pathak AK. CNS activity of *Calotropis gigantea* roots. J Ethnopharmacol. 2006; 106:142-145.
25. Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus*. Fitoterapia 2007; 78:25-28.
26. Malairajan P, Geetha G, Narasimhan S, Jessi Kala Veni K. Analgesic activity of some Indian medicinal plants. J. Ethnopharmacol. 2006; 19:425-428.
27. Latha RM, Geetha T, Varalakshmi P. Effect of *Vernonia cinerea* Less flower extract in adjuvant induced arthritis. Gen. Pharmacol. 1998; 31:601-606.
28. Haslem E. Plant polyphenols: vegetable tannins revisited-chemistry and pharmacology of natural products. Cambridge University Press. 1989, 169. (Original not seen).
29. Abdul –Lateef – Maloan, Abbas Mohammed Faraj, Abdulkhaliqsaleh Mahdy. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. J Phytopharmacology. 2012; 2(2):224-233.
30. Dormen HJD, Kosar M, Kahlos K, Holm Y, Hiltunen R. Antoioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varities and cultivars. J Agric Food Chem. 2003; 51:4563-4569.
31. Duh PD, Yen WJ, Du PC, Yen GC. Antioxidant activity of mung bean hulls. J Am Oil Chem Soc. 1997; 74:1059-1063.
32. Jayaprakasha GK, Girennavar B, Patil BS. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different *in vitro* model systems. Bioresour. Technol. 2008; 99(10):4484-4494.
33. Friedman M. Antibiotic activities of plant compounds against non-resistant and antibiotic resistant food borne human pathogens. ACS Symposium series. 2006; 931:167-183.
34. Scherrer R, Gerhardt P. Molecular sieving by the *Bacillus megaterium* cell wall and protoplast. J Bacteriol. 1971; 107:718-735.
35. Utpal Kumar Karmakar, Soma Pramanik, Samir Kumar Sadhu, Manik Chandra Shill, Subrata Kumar Biswas. Assessment of analgesic and antibacterial activity of *Premna tomentosa* Linn. (Family: Verbenaceae) Leaves. International Journal of Pharmaceutical Sciences and Research. 2011; 2(6):1430-1435.
36. Shahedur Rahman, Mc., Faizus Salehin Md, Abu Hena Mostofa Jamal, Anzana Parvin, Khasrul Alam Md. Antibacterial activity of *A, mexicana* L. against water borne microbes, 2011.