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PHYTOCHEMISTRY AND ANTIOXIDANT RELATED ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS

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ABSTRACT

The thrust on utilization of bioactive chemicals from plant sources is known since ancient times. Plants are rich in bioactive compounds and are used to cure a wide range of ailments, in the form of crude preparations in Ayurveda and Siddha, and Unani. The most commonly used four medicinal plants are selected for the present study. The plant phytochemicals are extracted using water and methanol separately and concentrated on water bath. *In vitro* antioxidant activity is done by DPPH radical scavenging activity and ABTS radical cation decolourization assay. In addition, the estimation of bacterial inhibition against five bacteria are done; three are gram positive and two are gram negative inorder to know the antioxidant activity related bacterial inhibition. The medicinal plants that are used showed maximum antioxidant property and maximum inhibition against the test organisms.

KEYWORDS: phytochemicals, analysis, antioxidant related antibacterial activity.

INTRODUCTION

In the world, the use of plant or plant parts as medicines is known since ages. The Indian medical systems such as Ayurveda, Siddha and Unani have been in existence for over several centuries. These traditional systems of medicine have served 70% of the rural and urban folk of developing countries for a variety of diseases.[1] All systems of medicine including Homeopathy and Allopathy used plants to prepare drugs, according to the World Health Organization report.[2] As per the plant list web site over 3,50,699 plant species have valid names which are existing or non existing on this earth. Among these existing plants, only 2% of plants have been explored and tested for their activity.

The plants are rich in bioactive compounds and used to cure a wide range of ailments, used in the form of crude preparations in Ayurveda and Siddha. These bioactive chemicals are concentrated in various parts of the plant, such as roots, stems, leaves, flower buds, flowers, fruits and seeds. It is also known in ayurvedic system of medicine the collection of plant materials for the drug preparation is done at a particular part of the day, either in the morning or in the evening. The reason is due to, the accumulation of bioactive chemicals in plant parts at a specified part of the day. This is of authors observation of people who are connected to ayurvedic system of collecting plants or plant parts either in the evening or in the early morning.

The majority of plants have antibiotic activity and they also possess large amounts of antioxidant free radicals. Generally antioxidants have medicinal value because the cell damage is reduced by nullifying the effect of free oyygen radicals in the body. Presently, there is greater abuse of antibiotics in developing countries and this resulted in increased antibiotic resistance among disease causing organisms. This kind of drug resistance of microorganisms has become a global concern.[3,4] The antioxidants have potency to control infective microorganisms.

Several of the plants that are investigated have been used in medicine since time immemorial. The ripened fibrous outer layer of fruits of Borassus flabellifer Linn., is edible either boiled or roasted and have antioxidant and neutraceutical properties.[5] Species of Gisekia phernaceoides Linn., has antibacterial antihelminthic property which is used in Ayurvedic system of medicine[6] and of Pedalium murex Linn., used in curing gonorrhoea and dysuria.[7] The ethanolic extract of fruits has nephro protective activity.[8] The member of Savadoraceae Salvadora persica Linn., used as a natural tooth brush and its branches have been promoted by World Health Organization for oral hygiene. It is said to be promising antiplaque, analgesic, antibacterial, antimycotic, diuretic and astringent.[9]

MATERIALS AND METHODS

These medicinal plant materials selected for our present work, are locally used to cure certain diseases especially in the state of West Bengal and also in other states. The plant parts that are used locally are the fruits of Borassus flabillifer Linn. (Arecaceae) the leaves and roots of Gisekia phernaceoides Linn. (Aizoaceae or Gisekiaceae) the fruits of Pedalium murex Linn. (Pedaliaceae) and flowers of Salvodora persica Linn. (Salvadoraceae).

The fruits of Borassus flabellifer, leaves and roots of Gisekia phernaceoides, the fruits of Pedalium murex and the flowers of Salvodora persica were dried in shade at a temperature between 21-30^0 C for 15-20days, and they were finely powdered and the extractions was carried out separately by the following procedures:

Preparation of extracts

Around 400 gms of powder was subjected to successive hot continuous extraction (soxhlet) with methanol and water. After the effective extraction, the solvents were distilled and the extract was then concentrated in water bath to avoid the decomposition of natural metabolites.

In-vitro antioxidant activity of DPPH and ABTS

DPPH (2,2-Diphenyl-1picryl hydrazyl) radical scavenging activity and ABTS (2,2$^{'}$-azinobis- (3-ethylbenzthiazoline- 6-sulfonic acid) radical cation decolourization assay was carried out.

Radical scavenging activity of DPPH

The effect of the plant extraction using DPPH was estimated according to the method of Ho et al (10). The principle of the reduction of DPPH free radical assay is that the antioxidants react with the stable DPPH radical and convert it into 1,1-diphenyl 2-picryl hydrazine. The ability of scavenging by DPPH radical is measured by a decrease in the absorbance.

Step I (preparation of stock solution)
Prepared 0.05Mμ solution of DPPH by mixing 9.8 μg of DPPH in 50 ml of ethanol and incubate at normal room temperature for 2-3 hrs.

Step-II (Preparation of plant extract of different dilutions)
The stock solution of 1 μg/ml of the plant extract is prepared in distilled water and diluted to get various concentrations (100-1000 μg/ml)

Step-III (Analysis of reaction mixture)
10 ml of the stock Solution dissolved in 10 ml of ethanol. This is taken as working solution. Mixed 1ml of the working solution with 1 ml of plant extract concentration (100-1000 μg/ml) prepared to a final volume of 1L. Incubated the mixture for 30 min. at room temperature.

ABTS radical decolourization Assay

The ABTS diammonium salt radical cation decolourization test was performed using spectrophotometric method. The principle of ABTS (2,2$^{'}$-azinobis-(3-ethylbenzthiazoline- 6-sulfonic acid) diammonium salt cation radical decolourization assay is that the antioxidant reacts with ABTS resulting in the decolourization of the ABTS radical in aqueous phase.

Step –I (preparation of stock solution)
Weighed 4 mg of ABTS and dissolved it in 100 ml of distilled water. Then 38mg of Potassium persulphate was taken and dissolved it in 1ml of distilled water from that 88μl of (K_2 S_2O_8) added to 100ml ABTS solution and kept it for overnight incubation at 4^{o}C.

Step II (working solution preparation)
The working ABTS reagent was prepared by diluting the stock solution with ethanol to give an absorbance of 0.7± 0.05 at 734 nm.

Step-III (Preparation of plant extract)
Stock solution of 1mg/ml of the plant extract is prepared in distilled water and diluted to get various concentration of (100-1000 μg/ml). The concentrations can further be increased depending upon the results.

Step-IV Mixed 1 ml of the working solution with 1ml of plant extract (100-1000 μg/ml) prepared to a final volume of 1ml.

Antibacterial activity

The plant parts have been selected and were dried at room temperature for over seven days and powdered. The powdered material was soaked separately in methyl alcohol for 48 hours and filtered using Whatman's filter paper. The filtrate obtained was concentrated under reduced pressure in rotary evaporator to obtain the crude extracts. The crude extracts were kept at 4^{o}C for further studies.

Anti microbial screening for five bacteria like Staphylococcus aureus (MTCC 3219), Escherichia coli (MTCC 298), Bacillus subtilis (MTCC 497), Klebsiella sp (MTCC 3378) and Pseudomonas aeruginosa (MTCC 2113) were performed. Both the disc diffusion and agar well diffusion methods were followed and results were tabulated in tables 5 and 6.

Preliminary qualitative phytochemical analysis of the plant extracts was performed by standard methods as mentioned below (11)

Alkaloids

Plant extracts were dissolved separately in 1% HCL and filtered. 1 ml of the filtratewas taken and a few drops of the following reagents were added and the respective colour changes indicated the presence of alkaloids.

Dragendorff's reagent–orange ppt.; Mayer's reagent-cream coloured ppt.; Hager's reagent–yellow ppt.; Wagnor's reagent-red-brown ppt.

Saponins
(Foam test) A small quantity of the residue was diluted with 20 ml of distilled water and shaken vigorously, formation of one cm layer of foam formation which is stable for 10 minutes indicated the presence of saponins.

Flavonoids
To the plant extract a few ml of con. H_2SO_4 was added, the precipitate formed was yellow or orange in colour indicates the presence of flavanoids.

Phenolics and tannins: A small amount of the extract was dissolved in distilled water and to it 10% of lead acetate was added, a white precipitate indicated the presence of phenolics and tannins.

DPPH result analysis was done as follows. After 20 minutes absorbance of reaction mixtures was recorded at 517nm. The blank (reaction mixtures without plant extract) served as control samples. The changes in the absorbance of the reaction mixtures were measured using a spectrophotometer and the percentage of scavenging or inhibition was calculated according to the formula.

$$\% \text{ of scavenging or inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

All procedures of DPPH were performed in dark.
Absorbance of reaction mixture for ABTS was recorded at 734nm. In all the experiments distilled water mixture without the plant extract (1ml of working solution) served as control. The changes in the absorbance of the reaction mixtures were measured using a spectrophotometer and the percentage of scavenging or inhibition was calculated as per the formula given above.

In vitro antioxidant activity of DPPH and ABTS free radical scavenging methods were calculated in different cons. like 100, 250, 500, 750 and 1000 µg /ml of the extracts of all the plant species investigated.

RESULTS AND DISCUSSION
The percentage of scavenging activity of the extracts of both aqueous and methanolic B. flabellifer has increased from lower to higher concentration. However, aqueous extract has less percentage when compared to methanolic extract both in DPPH scavenging activity and ABTS radical decolorization assay.
With regard to the antimicrobial activity according to Baur et al (12) the activity is referred as Resistance to bacteria, when the inhibition zone is less than 8mm and it is intermediate when it is greater than 11 mm. Majority of inhibitions are above 11mm and such bacteria are referred as susceptible to the plant extracts. The substances present in methanolic extracts of plants have the ability to inhibit the growth of bacteria. The microbial inhibition is observed and all the test organisms are susceptible to methanolic extracts of B.flabellifer in different concentrations.

In G.pharnaceoides the methanolic extracts have greater percentage of scavenging in DPPH and it is almost similar to decolourization assay in ABTS. The root and leaf extracts of G. pharnaceoides maximum inhibition of 22 mm of Staph. aureus, B. subtilis, E. coli, Klebsiella sp, and Pseudo. aeruginosa.

In P. murex and S.persica extracts of both aqueous and methanolic extracts have a increased trend of scavenging activity from lower to higher percentage of concentration. S.persica showed higher percentage of scavenging in DPPH extract than in aqueous extract. Similarly, ABTS has higher radical decolorization in methanolic extract than in aqueous extract.

P. murex extracts showed higher inhibition to Staph. aureus, B. subtilis, E. coli, Klebsiella sp., and Pseu.aeruginosa are all susceptible and S. persica has shown maximum inhibition of growth of all the bacteria investigated.

The antioxidant and antibacterial activity relationship have been observed in our study of different medicinal plants. The DPPH scavenging activity and ABTS radical decolourization assay and antibacterial activity have lot of similarity in their functioning. The antioxidant associated with antibacterial activity is relatively appreciable.

The plant based antibacterials have enormous therapeutic potential and more so they have lesser side effects that are often seen with synthetic antimicrobials (13). However, the present study of in vitro antibacterial and antioxidant evaluation of plants form a base for further phytochemical and pharmacological studies. The Bioactive chemicals like saponins, tannins, flavonoids, alkaloids, phenols, anthocyanins and triterpenes present in investigated species have therapeutic value.

Table -1 Effect of aqueous and methanolic extracts on DPPH and ABTS antioxidant method on Borassus flabellifer

Concentration of extract µg/ml	DPPH (%) inhibition Aq extract	DPPH (%) inhibition Methanolic extract	ABTS (%) inhibiton Aq extract	ABTS (%) inhibiton Methanolic extract
100	41.38± 0.25	38.2± 0.51	29.3±0.31	32.1±0.55
250	53.23±0.67	44.35±0.32	49.72±0.29	48.20±0.61
500	61.07±0.12	54.92±0.61	59.61±0.05	49.54±0.70
750	72.31±0.45	64.47±0.03	68.56±0.52	55.6±0.03
1000	86.74±0.23	72.26±0.21	79.80±0.31	64.52±0.92

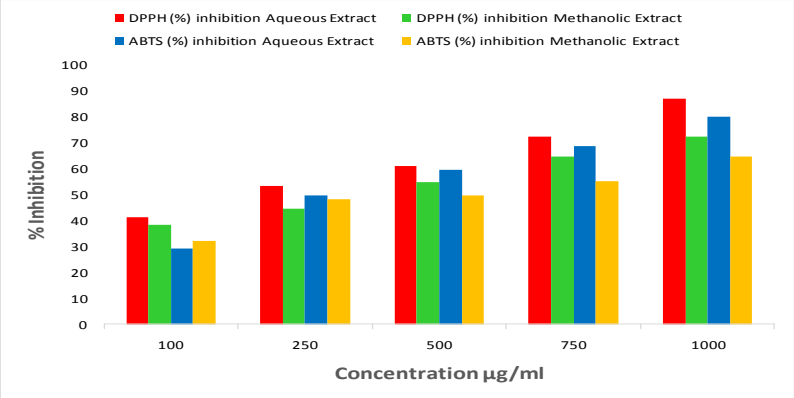


Table-2 Effect of aqueous and methanolic extracts on DPPH and ABTS antioxidant method of Gisekia phernaceoides

Concentration of extract µg/ml	DPPH (%) inhibition Aq extract	DPPH (%) inhibition Methanolic extract	ABTS (%) inhibiton Aq extract	ABTS (%) inhibiton Methanolic extract
100	53.11±0.21	68.32± 0.47	46.52±0.34	52.90±0.64
250	56.04±0.04	74.18±0.48	50.31±0.34	54.62±0.56
500	59.63±0.44	79.56±0.15	55.94±0.14	59.36±0.54
750	68.42±0.06	83.44±0.87	62.88±0.05	69.85±0.12
1000	75.86±0.56	87.88±0.27	69.67±0.65	74.09±0.23

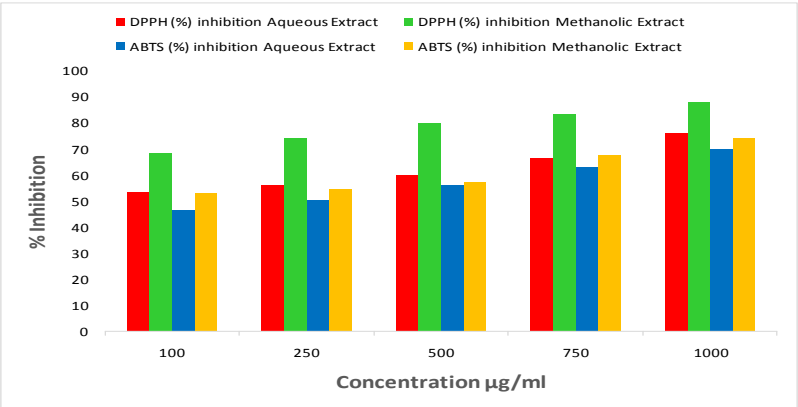


Table-3 Effect of aqueous and methanolic extracts on DPPH and ABTS antioxidant method of Pedalium murex

Concentration of extract µg/ml	DPPH (%) inhibition Aq extract	DPPH (%) inhibition Methanolic extract	ABTS (%) inhibiton Aq extract	ABTS (%) inhibiton Methanolic extract
100	25.76±0.34	44.07±0.27	20.23±0.21	31.25±0.45
250	40.22±0.08	57.53±0.56	34.33±0.26	40.50±0.51
500	53.02±0.33	59.42±0.07	44.56±0.05	49.32±0.02
750	67,55±0.5	61.98±0.86	64.48±0.06	70.36±0.04
1000	74,61±0.33	66. 08±0.07	73.87±0.36	79.48±0.42

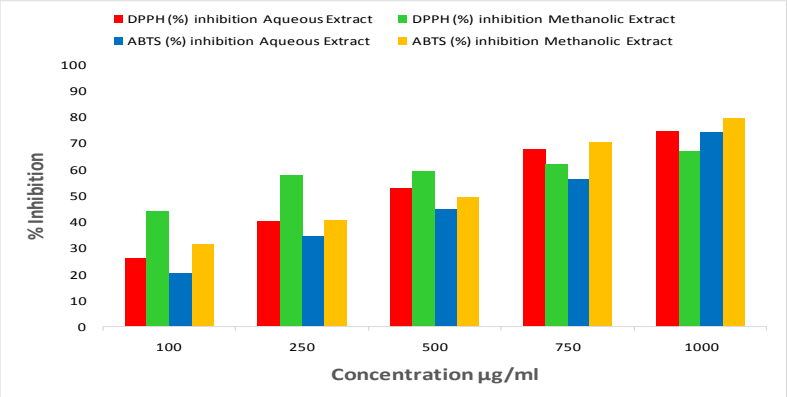
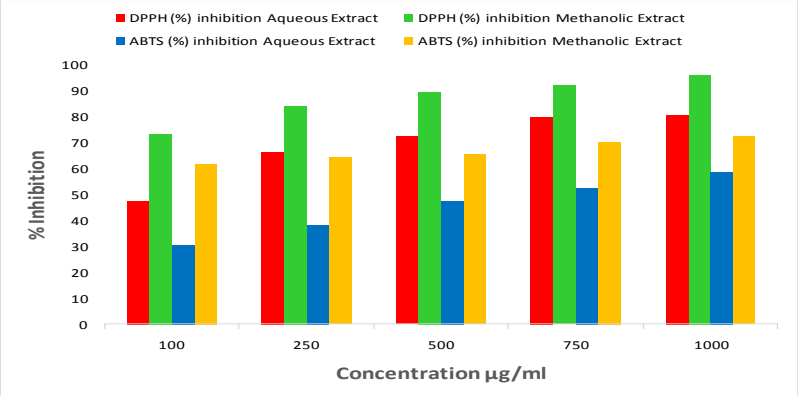


Table-4 Effect of aqueous and methanolic extracts on DPPH and ABTS antioxidant method of Salvodora persica

Concentration of extract µg/ml	DPPH (%) inhibition Aq extract	DPPH (%) inhibition Methanolic extract	ABTS (%) inhibiton Aq extract	ABTS (%) inhibiton Methanolic extract
100	41.26±0.31	73.42± 0.45	30.55±0.23	61.56±0.46
250	66.50±0.06	84.10±0.67	38.39±0.29	64.09±0.65
500	72.31±0.34	89.57±0.09	47.26±0.04	65.31±0.06
750	79.86±0.03	91.89±0.75	52.51±0.04	70.04±0.08
1000	80.71±0.36	96.03±0.06	58.77±0.56	72.37±0.32



Antimicrobial activity:

Table-5 Antibacterial activity of methanol extract of plants

Sl. No.	Plant name	Part used	Con. of sample	Zone of Inhibition in mm				
				Staph. aureus	B.subtilis	E.coli	Kleb. sp	Pseu. aeruginosa
1	B. flabellifer	fruit	100µL	19	17	19	15	NA
			125µL	17	15	14	18	NA
			150µL	10	16	13	12	NA
2	G. phernaceoides	Leaf	100µL	10	20	22	18	08
			125µL	12	15	22	20	09
			150µL	17	12	17	18	11
		Root	100µL	16	16	20	16	15
			125µL	15	19	20	16	11
			150µL	15	15	18	18	09
3	P.murex	Fruit	100µL	20	22	20	18	16
			125µL	18	22	21	22	18
			150µL	16	19	20	20	17
4	S. persica	Flower	100µL	21	18	19	18	16
			125µL	21	20	16	19	18
			150µL	19	20	15	19	17

Table-6 Phytochemical analysis of methanolic extract of plants

Phytochemicals	B.flabellifer	G.phernaceoides	P.murex	S.persica
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponins	_	+	_	+
Tannins	_	+	_	_
Anthocyanins	+	+	+	+
Triterpenes	+	+	+	+

CONCLUSION

The bioactive compounds present in plants have great therapeutic value. Four plant species, used in Ayurvedic medicine are selected to find the antimicrobial ability against three gram positive and two gram negative bacteria. The plant extracts are proved to be the source of antimicrobials.

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