

# Total phenolic content, Free radical scavenging and Antimicrobial activities of *Passiflora subpeltata* seeds

Shanmugam Saravanan and Thangaraj Parimelazhagan\*

Bioprospecting Laboratory, Department of Botany, Bharathiar University- 641 046, India.

---

## ARTICLE INFO

### Article history:

Received on: 08/02/2013

Revised on: 28/02/2013

Accepted on: 12/03/2013

Available online: 27/04/2013

### Key words:

*Passiflora subpeltata*; Free radicals; antioxidants; antimicrobial; DPPH; ABTS.

---

## ABSTRACT

The study aimed to investigate the antioxidant and antimicrobial activities of seeds from *Passiflora subpeltata*. The powdered seed material was subjected to successive solvent extraction in a soxhlet extractor. The antioxidant activities were analyzed the methods of DPPH<sup>•</sup>, ABTS<sup>•+</sup>, superoxide radical scavenging and metal chelating activity. The antimicrobial activities of seeds were performed by disc diffusion method. The acetone extract of *P. subpeltata* seed recorded highest total phenolic (340.70 mg GAE / g extract) and tannin (214.30 mg GAE / g extract) contents and possessed high antioxidant activity. The IC<sub>50</sub> value of DPPH was (26.37 µg/mL) and the scavenging activity of superoxide radical was (75.18%). *P. subpeltata* seeds also demonstrated an effective antibacterial property against seven pathogens and antifungal property against two pathogens tested. Therefore, the results suggest that the seeds of *P. subpeltata* have promising therapeutic value for natural antioxidant and antimicrobial properties.

---

## INTRODUCTION

During the recent years the food industries are facing a massive pressure due to deterioration of food and food products caused by the oxidation and microbial contaminations. The growth of microorganisms in food products are reported to cause intestinal disorders, vomiting and diarrhea (Loizzo *et al.*, 2010). It has been estimated that as many as 30% of people in industrialized countries suffer from a food-borne disease each year. Furthermore, the growing environmental pollution may lead to severe adverse food-borne diseases to more population (Friedman *et al.*, 2002). The growing resistance against antibiotics has created awareness among researchers to identify novel compounds from plant sources. Besides these, the synthetic antioxidants incorporated to prevent oxidation of food stuffs are found to cause severe adverse effects in human. Based on these reasons it is essential to develop naturally occurring products with antioxidant and antimicrobial activities. Natural products with potential bioactivity compounds have received major attention of many researchers.

They have been shown to be an excellent source for the development of food additives in food industry, and new drugs in medical fields (Zhang *et al.*, 2007). *Passiflora* commonly called as passion fruit plant, belonging to Passifloraceae family comprises nearly around 500 species is native to Tropical America and Brazil, and are widely distributed all over the world (Vanderplank, 1991). The species are very popular for their fruits (passion fruits) wherein almost 294 volatile compounds of interest have been isolated from them (Shibamoto *et al.*, 1990). Besides the fruits, the tea made from the leaves have been largely used as folk medicine in American and European countries as sedative, diuretic, and in the treatment of dysentery, hypertension, biliousness, asthma and skin diseases. The leaves of several species including *P. edulis*, *P. foetida* and other have been consumed as leafy vegetable. The pharmacological properties on the leaves of Passifloraceae include antioxidant, anti-inflammatory, analgesic, anti-spasmodic, anti-asthmatic, wormicidal, antimicrobial and sedative properties (Dhawan *et al.*, 2004). Among the several species, *Passiflora subpeltata* (Synonym –*P. calcarata*) commonly known as white passion flower, found growing along the higher altitudes. The plant bears attractive flowers and their fruits and leaves are edible. In Western Ghats of India, *P. subpeltata* leaves are widely consumed as a green leafy vegetable (Narayanan *et al.*, 2007).

---

\* Corresponding Author

T. Parimelazhagan

Bioprospecting Laboratory, Department of Botany,

Bharathiar University, Coimbatore-641 046 Tamil Nadu, India.

Tel: 0422 – 2428305.

A coumarin derivative, cyanogenic glycosides-barterin have been isolated in the *P. subpeltata* and the compound was found to possess sedative, anti-plasmodic and antimicrobial properties (Montgomery, 2008). Besides the above said compounds in the genus *Passiflora*, no reports are available revealing the antioxidant and antimicrobial potential of *P. subpeltata* seeds. Hence the present study aimed to investigate the free radical scavenging and antimicrobial properties of *P. subpeltata* seeds.

## MATERIALS AND METHODS

### Chemicals

Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), potassium persulfate, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), ferrous chloride, ammonium thiocyanate, ferrous ammonium sulfate, ethylenediamine tetracetic acid (EDTA) disodium salt, trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and ampicillin were obtained from Himedia and Sigma. Petroleum ether, chloroform, acetone, methanol and all the culture media were purchase from Himedia. All other reagents used were of highest purity and analytical grade.

### Preparation of extracts

The fruits of *P. subpeltata* were collected from Western Ghats of Tamil Nadu, India. The seed were separated from the fruits and shade dried, the seed was powered by mixer grinder the powdered material of seed was successively extracted in hot percolation method by using soxhlet extractor with different organic solvents like petroleum ether, chloroform, acetone and methanol. The different solvent extracts were concentrated using rotary vacuum evaporator (Yamato BO410, Japan) and then dried. The extracts thus obtained were used directly to assess the antioxidant potential through various *in vitro* assays.

### Quantification of total phenolics content tannins

The total phenolic content of different solvent extract of seed was quantified by Folin ciocalteu method described by Siddhuraju and Becker (2003). The analysis was performed in triplicate and the results were expressed as Gallic acid equivalents.

### Quantification of tannins content

Tannin content of plant extract was estimated the method described by Siddhuraju and Manian (2007). The plant extract was treated with the polyvinyl polypyrrolidone (PVPP), and the tannin content was quantified by Folin ciocalteu method. The analysis was performed in triplicate and the results were expressed as Gallic acid equivalents.

### Quantification of flavonoid content

The quantification of flavonoid content was determined according to the method described by Zhishen *et al.*, (1999). A 0.5

ml of different solvent extract of seed was mixed with 2 mL of distilled water and 0.15 ml of 5% NaNO<sub>2</sub> solution. After 6 min, 0.15 mL of 10% AlCl<sub>3</sub> solution was added and allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate and the results were expressed as rutin equivalent (RE).

### Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant capacity of different solvent extracts of *P. subpeltata* seed was estimated by the method described by Pulido *et al.*, (2000). Freshly prepared FRAP reagent (900 µL) was taken and incubated at 37 °C, then mixed with 90 µL of distilled water and 0.1 ml of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 °C for 30 min in a water bath. At the end of incubation, the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2,000 µmol/L, (FeSO<sub>4</sub>·7H<sub>2</sub>O) were used for the preparation of the calibration curve. The FRAP value is expressed as mmol/L Fe (II) equivalent/mg extract.

### Metal chelating activity

The chelation of metal ions by different solvent extracts of *P. subpeltata* seed were done as proposed by the method Dinis *et al.*, (1994). Briefly the extract samples (100 µL) were added to a solution of 2 mmol/L FeCl<sub>2</sub> (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

### DPPH radical scavenging activity

Radical scavenging activity of different solvent extracts of *P. subpeltata* seed was measured by DPPH radical scavenging method, described by Blois, (1958). IC<sub>50</sub> values of the extract i.e., concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

### ABTS cation radical scavenging activity

The total antioxidant activity of the plant extract was measured by ABTS radical cation decolorization assay according to the method of Re *et al.*, (2000). ABTS radical was generated by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Proceeding to assay, this ABTS was diluted with ethanol (about 1: 89 v/v) and equilibrated at 30 °C to give an absorbance at 734 nm of 0.70 ± 0.02. The concentration of extracts that produced between 20-80% inhibitions of the blank absorbance was determined and

adapted. After the addition of 1 mL of diluted ABTS<sup>++</sup> solution to 20 µL of seed or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was measured at 30 °C exactly 30 min after the initial mixing. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µM/g sample extract as dry matter.

### Superoxide radical scavenging activity

The assay was based on the capacity of the plant extract to inhibit formazan formation by scavenging superoxide radicals generated in riboflavin–light–NBT system as described by Beauchamp and Fridovich, (1971). The analysis was performed in triplicate. The inhibition percentage of superoxide radical generation was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

### Antimicrobial activity

Different solvent extract of *P. subpeltata* seed were separately tested adjacent to a group of pathogenic micro organism including Gram-positive bacteria's *Streptococcus faecalis*, *Streptococcus pyogenes*, *Bacillus subtilis* and Gram-negative bacteria's *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Salmonella typhi A*, *Salmonella typhi B* and pathogenic fungus *Candida albicans* and *Aspergillus niger*. The pure bacterial and fungal strains were obtained from the PSG Medical College and Hospital, Coimbatore, Tamil Nadu, India. Bacterial strains were cultured for 24 h at 37°C on nutrient agar (NA, Himedia, India), while the fungal strains were cultured for 24 to 48 h at 37°C using potato dextrose agar (PDA, Himedia, India).

### Disc diffusion method

Different solvent extract of *P. subpeltata* seed were dissolved in respective solvents, to a final concentration of 10mg/mL. The antimicrobial test was carried out by NCCLS disc diffusion method (1971). Briefly, 100 µL of suspension containing approximately 10<sup>8</sup> colony form units (CFU)/mL of bacterial cells and 10<sup>4</sup> cells/mL of fungal cells were spread on to nutrient agar (NA) and potato dextrose agar (PDA) medium, respectively. For the antimicrobial test, paper discs (6 mm diameter) were separately impregnated with 15 µL of the 300 mg/mL plant extracts (4500 µg/disc) and placed on the inoculated agar. For the positive control, paper discs were impregnated with (15 µL) of ampicillin (4500 µg/disc) dissolved in both types of solvents used. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. For the antifungal test, *P. subpeltata* seed extracts were dissolved in their respective solvents to a final concentration of 10 mg/mL. Paper discs were impregnated with 30 µL of the 10 mg/mL extracts (300 µg/disc) and placed onto the inoculated agar. As a positive control, ampicillin was prepared by dissolving it in both solvents used to a final concentration of 1 mg/mL. Paper discs for the positive control were impregnated with 30 µL of ampicillin (30 µg/disc). Negative controls were prepared

again using the same solvents employed to dissolve the plant extracts. Plates were incubated at 37° C for 18-24 h for bacterial strains and for 24-48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth inhibition zone (IZ) in millimeters (including disc diameter of 6 mm) for the test organisms compared to controls.

### Statistical analysis

The results were expressed as mean ± standard error mean (SEM). Statistical analysis was carried out by analysis of variance (ANOVA) followed by Duncan's test. *P* < 0.001, 0.01 and *P* < 0.05 were considered as indicative of significance as compared to the control group using the statistical software, SPSS, version-17.0.

## RESULT AND DISCUSSION

### Total phenolics, tannin and flavonoid contents

The results of total phenolics, tannin and flavonoid content of *P. subpeltata* seed are presented in table 1. The acetone extract of seed recorded highest amount of total phenolic (340.70 mg GAE/g extract), tannin (214.30 mg GAE/g extract) and flavonoid (287.33 mg RE/g extract) content. However, the methanol, petroleum ether and chloroform extracts also recorded moderate amounts of total phenolics, tannin and flavonoid contents. Enormous integers of *in vitro* methods have been performed to determine the effectiveness of natural antioxidants whichever as pure compounds or as crude plant extracts. Antioxidant compounds act by several mechanisms. The polyphenols and the diverse group of phenolic compounds (flavonoids, anthocyanins, phenolic acids, etc.) derived from plants acquire an idyllic structural chemistry for scavenging free radicals. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors from the ability of the polyphenols which derive radical to stabilize and delocalize the unpaired electron (chain-breaking function) (Chanda *et al.*, 2009). The total amount of phenolic, tannin and flavonoid content present in seed of *P. subpeltata* were found to be higher in the acetone extract. Calderon *et al.*, (2011) reported earlier the *P. mollissima* fruit contains (635±2.71mg GAEs/100g extract) of phenolics. When compared to *P. mollissima* the seed of *P. subpeltata* possess an incredible amount of phenolic contents (340.70 mg GAE/g extract).

**Table. 1:** Total phenolic tannin and flavonoid contents of *P. subpeltata* seed.

S.No	Sample	Total phenolics (GAE mg/ g extract)	Tannin(GAE mg/ g extract)	Flavonoids(RE mg/g extract)
1	Petroleum Ether	122.90c ±1.89	31.90c ±1.32	228.33b ±2.31
2	Chloroform	137.90b ±3.52	67.90b ±5.02	128.00c ±1.00
3	Acetone	340.70a ±4.95	214.30a ±2.95	287.33a ±1.08
4	Methanol	115.70d ±3.60	60.23b ±2.93	231.31b ±1.50

Values are means of three independent analyses ± standard deviation (n = 3).

Mean values followed by different superscript letters indicate significant statistical difference (a - *P*<0.001, b - *P*<0.01 and c - *P*<0.05).

### Ferric reducing antioxidant power (FRAP) assay

The results of ferric reducing antioxidant power assay are shown in Table 2. Among the different solvent extracts of *P. subpeltata* the acetone extract followed by methanol extract showed the maximum amount of reduction (63.03 and 45.20 mmol Fe (II)/ mg extract, respectively). This method is ideal to determine the capability of antioxidants to decrease the ferric iron. It is based on the multifaceted form of ferric iron and 2, 3, 5-triphenyl-1, 3, 4 triaza-2- azoniacyclopenta-1,4-diene chloride (TPTZ) to the ferrous form. This lowering is monitored through measuring the change in absorption at 593nm, using the spectrophotometer.

**Table 2:** FRAP, Metal chelating and ABTS radical scavenging activity of *P. subpeltata* seed.

S. No	Sample	FRAP(mmol Fe (II)/ mg extract)	Metal chelating Activity (mg EDTA/g extract)	ABTS <sup>+</sup> ( $\mu$ M trolox equi/g extract)
1.	Petroleum Ether	30.11 <sup>d</sup> $\pm$ 0.78	45.22 <sup>d</sup> $\pm$ 1.55	1887.69 <sup>d</sup> $\pm$ 12.59
2.	Chloroform	28.12 <sup>c</sup> $\pm$ 0.28	39.15 <sup>c</sup> $\pm$ 1.78	1942.24 <sup>c</sup> $\pm$ 8.21
3.	Acetone	63.03 <sup>a</sup> $\pm$ 0.10	144.23 <sup>a</sup> $\pm$ 0.20	3276.98 <sup>a</sup> $\pm$ 3.55
4.	Methanol	45.20 <sup>b</sup> $\pm$ 1.15	123.29 <sup>b</sup> $\pm$ 0.10	3074.23 <sup>b</sup> $\pm$ 5.81

Values are means of three independent analyses  $\pm$  standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference (<sup>a</sup> -  $P < 0.001$ , <sup>b</sup> -  $P < 0.01$  and <sup>c</sup> -  $P < 0.05$ ).

### Metal chelating activity

Elemental species such as ferrous iron (Fe<sup>2+</sup>) can facilitate the production of ROS within animal and human systems; hence, the ability of substances to chelate iron can be a valuable antioxidant capability. Iron, in nature, can be found as either ferrous (Fe<sup>2+</sup>) or ferric ion (Fe<sup>3+</sup>), with the latter form of ferric ion predominating in foods. Ferrous ions (Fe<sup>2+</sup>) chelation may render important antioxidative effects by retarding metal-catalyzed oxidation. The ferrous iron chelating activity of seed of *P. subpeltata* are shown in table 2.

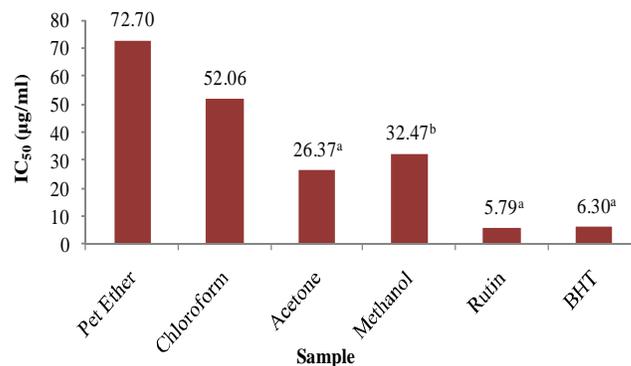
All the extracts were capable of chelating ferrous ion effectively. Ferrous ions (Fe<sup>2+</sup>) are the most powerful pro-oxidants among the various species of metal ions. Minimizing ferrous (Fe<sup>2+</sup>) ions may afford protection against oxidative damage by inhibiting production of ROS and lipid peroxidation. Ferrozine can quantitatively form complexes with Fe<sup>2+</sup> in this method. In the presence of chelating agents the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimating the metal chelating activity of the coexisting chelator. Lower absorbance indicates higher metal chelating activity.

Chelation of Metal iron is an important antioxidant property; therefore the seed extract was assessed for its ability to struggle with ferrozine for ferrous Fe<sup>2+</sup> in the solution. In this assay, the seed interfered with the formation of ferrous and ferrozine complex suggesting that they have chelating activity and are capable of capturing ferrous iron before ferrozine. Especially the acetone extract reduced the red colour complex immediately and gave the higher chelating activity (144.23 mgEDTA/g extract)

followed by the methanol extract which also produced the better amount of activity.

### DPPH radical scavenging activity

The IC<sub>50</sub> values of different solvent extract of seed are shown in Figure 1. The result suggested that the acetone extract of seed of *P. subpeltata* exhibit highest activity (IC<sub>50</sub> 26.37  $\mu$ g/ml) followed by the methanol extract which also gave better result (IC<sub>50</sub> 32.47  $\mu$ g/ml). DPPH assay is the common spectrophotometric method for analyzing the antioxidant property and it involves a hydrogen atom transfer process (Kaviarasan et al., 2007). The acetone extract of seed of *P. subpeltata* which contains the enormous amount of hydrogen donor molecules (IC<sub>50</sub> 26.37  $\mu$ g/ml) found from the reduced production of radicals and their ability to decolorize the purple colour of the DPPH. Naturally, fruits and vegetables comprise a huge amount of antioxidants which diminish the radicals and protect the body cells. Hence the results supported that the seed of *P. subpeltata* exhibit excellent antioxidant activity. Several studies have found the phenolic contents of plant extracts contribute to the excellent antioxidant/radical scavenging ability (Srinivasa Rao et al., 2010). Therefore, our finding revealed that the antioxidant activity of *P. subpeltata* seed might be attributed to at least in part of the phenolic compounds.



**Fig. 1:** DPPH radical scavenging activity of *Passiflora subpeltata* seed. Values are mean of different aliquot determination (n=3)  $\pm$  standard deviation. Mean values followed by different superscript letters indicate significant statistical difference (<sup>a</sup> -  $P < 0.001$ , <sup>b</sup> -  $P < 0.01$ ).

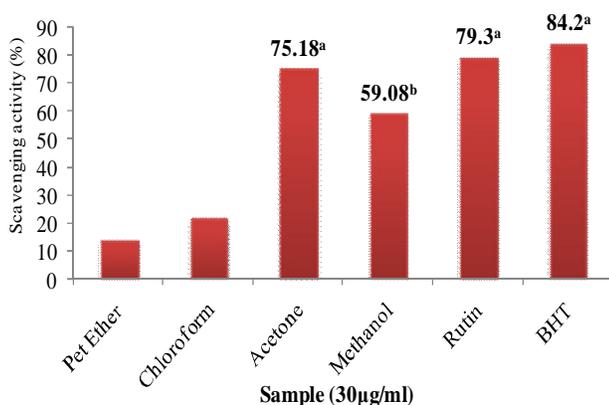
### ABTS cation radical scavenging activity

The efficiency of ABTS cation radical scavenging activity expressed as Trolox equivalent in different solvent extract of seed of *P. subpeltata* is shown in table 2. The results obtained in the present investigation revealed that the acetone extract of seed possess an utmost activity (3276.98  $\mu$ M trolox equi/g extract). ABTS<sup>+</sup> are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involves the hydrogen atom transfer; the reaction with ABTS radicals involves the electron transfer process (Kaviarasan et al., 2007). The experiment is carried out using the decolorization assay, which involves the production of the ABTS chromophore by the oxidation of ABTS with potassium persulphate. It is applicable to both hydrophilic and lipophilic compounds. The seed extract reacted with the ABTS

solution and donate more electrons to reduce the colour of the solution which gives the higher amount of Trolox equivalent values implies a greater antioxidant activity. Earlier study reported that the species of *P. foetida* fruit peel, flower, leaves and seed shows better ABTS cation radical scavenging activity (Sasikala *et al.*, 2011).

### Superoxide radical scavenging activity

Superoxide radical scavenging assay estimated based on the capacity of the samples to improve the aerobic photochemical reduction of nitroblue tetrazolium (NBT) in the presence of riboflavin. All solvent extracts of seed of *P. subpeltata* showed significant scavenging activity on the superoxide radicals in a dose dependent manner (Figure 2).



**Fig. 2:** Superoxide radical scavenging activity of *P. subpeltata* seed. Values are mean of triplicate determination (n=3) ± standard deviation. Mean values followed by different superscript letters indicate significant statistical difference (<sup>a</sup> -  $P < 0.001$  and <sup>b</sup> -  $P < 0.01$ ).

Ability of acetone extract to scavenge superoxide radicals (75.18%) is appreciable when compared to Rutin (79.3%) and BHT (84.2%). Superoxide is also one of the most harmful radical and its scavenging is necessary because it acts as the precursor for other major ROS like hydrogen peroxide, hydroxyl and singlet oxygen. Numerous biological reactions generate superoxide radical which is a highly toxic species (Kannat *et al.*, 2007). Since, the acetone and methanol extracts obtained by the soxhlet extraction of seed of *P. subpeltata* showed appreciable percentage of scavenging activity against superoxide radical, it can be used against adverse effects caused by superoxide radical in the body. Phenolic compounds are a category of phytonutrients with strong antioxidant properties and the relationships between

phenolic content and antioxidant activity have been reported earlier (Newell *et al.*, 2010). Therefore the higher amount of phenolic content present in acetone extract of seed of *P. subpeltata* might have contributed to an effective superoxide radical scavenging property.

### Antimicrobial activity

#### Disc diffusion method

Different solvent extracts of *P. subpeltata* seed exhibited varying antimicrobial activity, as shown by the growth inhibition zones (IZ) (Table 3).

The greater inhibition zone, indicate greater antimicrobial activity. The results from the disc diffusion method indicate that the tested *P. subpeltata* seed extracts had comparable antibacterial effects against both Gram-positive and Gram-negative bacteria. Furthermore, it can be seen from Table 3, that the acetone extract followed by methanol extract showed more inhibition against most of the bacteria. However, against *S. typhi* B and *K. pneumoniae*, the acetone extract showed better antibacterial activities (27 and 23 mm, respectively) than the methanol extract (14 and 17 mm, respectively). The result from the disc diffusion method against the fungal strains is shown in Table 3.

It can be seen that both acetone and methanol extracts *P. subpeltata* seed exhibited (13.23 and 15.21 mm) excellent antifungal activity in *A. niger*. The results were comparable with the standard drug, Ampicillin and Fluconazole. Results from the antimicrobial study suggest that phenolic compounds are responsible for the antibacterial and antifungal activities of different extract of *P. subpeltata* seed. Numerous works have reported the antibacterial effects of these metabolites against a wide range of bacteria (Rodríguez *et al.*, 2009). It has been reported that hydrolyzable tannins have potent antibacterial effects on various bacteria including *B. subtilis* (Tian *et al.*, 2009). Phenolic compounds can act at two different levels: the cell membrane and cell wall of the microorganisms. They can interact with the membrane proteins of bacteria by means of hydrogen bonding through their hydroxyl groups which can result in changes in membrane permeability and cause cell destruction. They can also penetrate into bacterial cells and coagulate cell content (Sengul *et al.*, 2009). Phenolic compounds are known to be synthesized by plants in response to microbial infection; it is therefore logical that they have been found *in vitro* to be effective antimicrobial substances against a wide array of micro-organisms (Makhlouf *et al.*, 2013).

**Table 3:** Antimicrobial activity of *P. subpeltata* seed.

S.No	Microorganism	Inhibition Zone (mm) of <i>P. subpeltata</i> seed				
		Ampicillin	Fluconazole	Chloroform	Acetone	Methanol
1	<i>S. faecalis</i>	11.65±1.03	-	7.93±0.52	10.00±1.60	12.67±1.58
2	<i>S. pyogenes</i>	16.70±0.12*	-	6.65±0.59	13.00±1.23	11.33±0.58
3	<i>B. subtilis</i>	17.76±1.21*	-	8.33±0.50	11.33±1.56	13.67±1.43
4	<i>S. paratyphi</i>	25.01±1.50*	-	5.67±1.33	21.67±1.52*	18.00±1.76*
5	<i>S. typhi A</i>	27.01±0.12*	-	8.05±1.20	23.67±1.53*	14.00±1.02*
6	<i>S. typhi B</i>	29.30±1.00*	-	5.67±1.15	27.00±1.00*	12.00±1.25*
7	<i>K. pneumoniae</i>	29.21±1.65*	-	6.00±1.06	23.00±1.00*	17.00±1.00*
8	<i>C. albicans</i>	-	13.30±1.24*	7.80±0.50	13.05±1.34*	12.34±1.90*
9	<i>A. niger</i>	-	16.50±0.38*	9.34±0.42	15.21±0.12*	13.23±0.23*

Values are means of three independent analyses ± standard deviation (n = 3). Mean values followed by different superscript letters indicate significant statistical difference (<sup>\*</sup> -  $P < 0.001$ ).

This study shows that the acetone extract of *P. subpeltata* seed contained higher phenolic and tannin contents and were active against two fungal strain (*Candida albicans* and *Aspergillus niger*), three Gram-positive strains (*Streptococcus faecalis*, *Streptococcus pyogenes*, *Bacillus subtilis*) and four Gram-negative bacteria (*Salmonella paratyphi*, *Salmonella typhi* A, *Salmonella typhi* B and *Klebsiella pneumoniae*).

## CONCLUSION

From the observed results it could be concluded that the acetone extract of *P. subpeltata* seed may be a probable source of natural antioxidant revealed from various *in vitro* assays.

The results of the antimicrobial experiments suggest that *P. subpeltata* seed acetone extract may be used as an alternative source for the treatment microbial infections.

## ACKNOWLEDGEMENT

The authors are very grateful to the University Grants Commission, New Delhi, India. (UGC letter No: F.No.37-95/2009 (SR)) for financial support of this major research project work.

## REFERENCE

- Loizzo MR., Tundis R., Chandrika UG., Abeysekera AM., Menichini F and Frega NG. Antioxidant and antibacterial activities on food-borne pathogens of *Artocarpus heterophyllus* Lam. (Moraceae) leaves extracts. *J. Food. Sci.* 2010; 75: M291-M295.
- Friedman M., Henika PR and Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food. Prot.* 2002; 65: 1545-1560.
- Zhang M., Cui SW., Cheung PCK and Wang Q. Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends. Food. Sci. Technol.* 2007; 18: 4-19.
- Vanderplank J. *Passion Flowers*. 1<sup>st</sup> Ed. Cassel Publishers Limited, London (1991) pp. 76.
- Shibamoto T and Tang CS. Minor tropical fruits Mango, papaya, passion fruit and guava. In: I. D. Morton, A. J. Macleod (Eds.), *Food favours, part C. The favour of fruits*. Amsterdam, The Netherlands, Elsevier (1990) p. 221-280.
- Dhawan K., Dhawan S and Sharma A. *Passiflora*: a review update. *J. Ethnopharmacol.* 2004; 94:1-23.
- Ratheesh Narayanan MK and Anil Kumar N. Gendered knowledge and changing trends in utilization of wild edible greens in Western Ghats, India. *Ind. Trad. Knowledge.* 2007; 6(1): 204-216.
- Pam Montgomery. *Plant Spirit Healing A Guide to Working with Plant Consciousness*. Published on: Bear and Company Rochester, Vermont (2008).
- Siddhuraju P and Becker K. Studies on antioxidant activities of mucuna seed (*Mucuna pruriens* var. utilis) extracts and certain non-protein amino/imino acids through *in vitro* models. *J. Sci. Food. Agric.* 2003 83: 1517-1524.
- Siddhuraju P and Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. *Food. Chem.* 2007; 105: 950-958.
- Zhishen J., Mengcheng T and Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. *Food. Chem.* 1999; 64: 555-559.

Pulido R., Bravo L., Sauro-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food. Chem.* 2000; 48: 3396-3402.

Dinis TCP., Madeira VMC and Almeida LM. Action of phenolic derivatives (acetoaminophen, salicylate, and 5 aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch. Biochem. Biophys.* 1994; 315: 161-169.

Blois MS. Antioxidants determination by the use of a stable free radical. *Nature.* 1958; 26: 1199-1200.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M and Rice- Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free. Radic. Biol. Med.* 2000; 26: 1231-1237.

Beauchamp C and Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.* 1971; 44: 276-277.

NCCLS, Wayne PA. Performance standards for antimicrobial disc susceptibility test. 6th ed. National Committee for Clinical Laboratory Standards; Approved Standard (1997) M2-A6.

Chanda S and Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. *Afr. J. Microbiol. Res.* 2009; 3(13): 981-996.

Calderón JC., Jaimes LC., Hernández EG., Villanova BG. Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food. research. Inter.* 2011; 44(7), 2047-2053.

Kaviarasan S., Naik GH., Gangabhairathi R., Anuradha CV., Priyadarsini KI. *In vitro* studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. *Food. Chem.* 2007; 103: 31-37.

Srinivasa Rao K., Chaudhury PK and Pradhan A. Evaluation of anti-oxidant activities and total phenolic content of *Chromolaena odorata*. *Food. Chem. Toxicol.* 2010; 48: 729-732.

Sasikala V, Saravana S and Parimelazhagan T. Evaluation of antioxidant potential of different parts of wild edible plant *Passiflora foetida* L. *J. Applied. Pharm. Sci.* 2011; 01(04): 89-96.

Kannat SR., Chander R and Sharma A. Antioxidant potential of mint (*Menthaspicata* L.) in radiation-processed lamb meat. *Food. Chem.* 2007; 600: 451-458.

Newell AM., Yousef GG., Lila MA., Ramírez-Mares MV., De Mejia EG. Comparative *in vitro* bioactivities of tea extracts from six species of *Ardisia* and their effect on growth inhibition of HepG2 cells. *J. Ethnopharmacol.* 2010; 130: 536-544.

Rodríguez H., Curiel JA., Landete JM., De las Rivas B., De Felipe FL., Gómez- Cordovés C., Mancheno JM and Munoz R. Food phenolics and lactic acid bacteria. *Inter. J. Food. Microbiol.* 2009; 132: 79-90.

Tian F., Li B., Ji B., Zhang G and Luo Y. Identification and structure-activity relationship of gallotannins separated from *Galla chinensis*. *LWT-Food. Sci. Technol.* 2009; 42: 1289-1295.

Sengul M., Yildiz H., Gungor N., Cetin B., Eser Z and Ercili S. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak. J. Pharm. Sci.* 2009; 22: 102-106.

Makhlouf BL., Slimani S and Madani K. Total phenolic content, antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Ind Crops Prod* 2013; 41: 85- 89.

### How to cite this article:

Shanmugam Saravanan and Thangaraj Parimelazhagan. Total phenolic content, Free radical scavenging and Antimicrobial activities of *Passiflora subpeltata* seeds. *J App Pharm Sci*, 2013; 3 (04): 067-072.