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## Free radical scavenging activity, phenol and flavonoid contents of various solvent extracts from the *Psydrax dicoccos* (Gaertn)

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### Abstract

**Aim:** The present study was to determine the *in vitro* antioxidant activity of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Psydrax dicoccos* (Wall. & Arn.) Swingle.

**Methods:** The different extracts of *P. dicoccos* leaves were antioxidant potential by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2, 2-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS<sup>+</sup>), Hydrogen peroxide scavenging, Superoxide anions scavenging, Hydroxyl radical scavenging, Ferric reducing antioxidant power, Total antioxidant activity (Phosphomolybdic acid) IC<sub>50</sub> values were calculated compare to standard antioxidant for L- ascorbic acid, (BHT) butylated hydroxytoluene and gallic acid.

**Results:** The methanol extract showed the highest phenol and flavonoid contents were also investigated. Among the different extracts methanol extract of *Psydrax dicoccos* had significantly higher. The total phenol (5.05 ± 0.76) mg/ml gallic acid equivalent (GAE/g) and flavonoids (6.72 ± 0.13) mg/ml quercetin equivalent (QE/g) were found to be higher in methanol extract of *Psydrax dicoccos*.

**Conclusion:** The results of the study revealed that the methanol extract of *P. dicoccos* can be an interesting source of natural antioxidants with their potential use in novel bioactive compounds.

**Keywords:** *Psydrax dicoccos*, antioxidant activity, total phenol and flavonoid contents

### 1. Introduction

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products used in the traditional systems of medicine [1]. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately, 20 per cent of the plants found in the world have been submitted to pharmaceutical or biological tests and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources [2].

Medicinal plants are finding their way into pharmaceuticals, cosmetics and nutraceuticals. In pharmaceutical field, medicinal plants are mostly used for the wide range of substances present in plants, which have been used to treat chronic as well as infectious diseases [3]. *Psydrax dicoccos* belongs to the family Rubiaceae and distributed in all parts of tropical and sub-tropical region of India. All parts of the plant have been recognized to have medicinal properties. *Psydrax dicoccos* is common names in Tamil are Nanjul, 'Nallamandharam'. Plant possesses antipyretic activity. In India, bark is used as febrifuge and also applied as plasters. The decoction of roots is used internally for treating diarrhoea. Bark powder boiled with sesame oil is used externally for rheumatic pains [4].

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical scavenging mechanism as part of their activity [5, 6].

Thus, antioxidant compounds which are responsible for reducing cellular oxidative stress could be isolated and then serve as leads for the development of novel drugs for the prevention and treatment of many human diseases [7]. In addition, it has recently been shown

that some anti-inflammatory compounds have an antioxidant effect or radical scavenging mechanism as part of their activity [8, 9], and therefore the research into the determination of natural antioxidant sources is very important to promote public health.

A number of flavonoids have been shown to suppress carcinogenesis in various animal models [10]. The antioxidant property of flavonoids was the first mechanism of action studied, in particular with regard to their protective effect against cardiovascular diseases. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals [11] that are probably involved in several diseases. Hence, present study *Psydrax dicoccos* leaves different extracts were subjected to antioxidant potential, phenol and flavanoid contents.

## 2. Materials and methods

### 2.1 Collection of Plant material

The fresh leaves of *Psydrax dicoccos* (Rubiaceae) were collected from Silambur (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. During the months from March to April 2014. The specimens were deposited in the Herbarium of Department of Botany, Annamalai University, Annamalai Nagar. Collected leaves were initially washed with water, then surface sterilized with disinfectant solution of 10 % sodium hypochlorite solution and finally rinsed with sterile distilled water and shade dried under room temperature and grounded in to a coarse powder. One hundred grams of coarse powder was extracted with different organic solvents like petroleum ether, chloroform, ethyl acetate and methanol for 8 hours using

Soxhlet apparatus and the solvents were evaporated under vacuum in a rotary evaporator (Heidolph, Germany) and the dried powder was stored at 4°C for further use.

### 2.2 Preparation of extraction

One hundred grams of powdered material of leaf, samples were extracted in a Soxhlet apparatus for 8 hours with different solvents system like petroleum ether, chloroform, ethyl acetate and methanol. The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporator (Heidolph, Germany) under reduced pressure at 40 °C and the crude extracts were kept at 4°C in refrigerator for further analysis.

### 2.3 DPPH (1, 1- diphenyl – 2 – picrylhydrazyl hydrate radical scavenging activity)

The DPPH radical scavenging activities of the different extracts of *P. dicoccos* was evaluated by the method of Blois [12]. A different extracts of leaf samples (0.1ml) at various concentrations (125, 250, 500 and 1000 µg/ml) was mixed with 1ml of 0.2mM DPPH dissolved in methanol. The reaction mixture was incubated for 20 min at 28 °C in the dark. The control contained all the reagents without the leaf sample and was used as blank. The DPPH radical scavenging activities were determined by measuring the absorbance at 517 nm using a Spectrophotometer (Hitachi U-20). Vitamin C was used as positive control. The antioxidant activities of plant extracts were expressed as IC<sub>50</sub>, which was defined as the concentration (µg/ml) of extracts required to inhibit the formation of DPPH radicals by 50 per cent. The DPPH radical concentration was calculated using the following equation.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 2.4 ABTS<sup>+</sup> Scavenging effects (2, 2-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid)

The antioxidant effect of the different crude extracts of *P. dicoccos* was evaluated by the method of Re *et al.* [13]. ABTS<sup>+</sup> radical cations (ABTS<sup>+</sup>) were produced by reacting ABTS<sup>+</sup> solution 7mM with 2.45mM potassium persulfate. The mixture was incubated at room temperature in the dark for 12 to 16 h to yield a dark-colored solution containing

ABTS<sup>+</sup> radicals and diluted. The different concentration of (125, 250, 500 and 1000 µg/ml) extracts were added to 1 ml of ABTS<sup>+</sup> solution. The absorbance was read at 734 nm after 6 min in a Spectrophotometer (Hitachi U-20). BHT was used as the standard. Appropriate solvents blanks were run in each assay. All determinations were carried out in triplicate and the per cent of inhibition was calculated using the formula.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### 2.5 Hydrogen peroxide scavenging effects

The ability of the different extracts of leaf samples were evaluated by the method of Ruch *et al.* [14]. A solution of H<sub>2</sub>O<sub>2</sub> (40mM) was prepared in phosphate buffer. Different crude extracts at the various concentrations of (125, 250, 500 and 1000 µg/ml) were added to H<sub>2</sub>O<sub>2</sub> solution (0.6 ml)

and the total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm in a Spectrophotometer (Hitachi U-20). A blank solution containing phosphate buffer, Vitamin C was used as positive control. The extent of H<sub>2</sub>O<sub>2</sub> scavenging of the leaf extracts were calculated using the formula.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 2.6 Superoxide anions scavenging activity

The superoxide anions scavenging ability of the different leaf crude extracts were assessed by the method of Winterbourn *et al.* [15]. Superoxide anions were generated in leaf samples that contained in 3.0 ml, 0.02 ml different crude extracts at the concentration of (125, 250, 500 and 1000 µg/ml) 0.2 ml of EDTA, 0.1 ml of NBT, 0.05 ml of riboflavin and 2.64 ml of phosphate buffer. The control

tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a Spectrophotometer (Hitachi U-20). Vitamin C was used as positive control. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activities.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 2.7 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described by Elizabeth and Rao [16]. The reaction mixture contained 0.1 ml of deoxyribose, 0.1 ml of FeCl<sub>3</sub>, 0.1 ml of EDTA, 0.1 ml of H<sub>2</sub>O<sub>2</sub>, 0.1 ml of ascorbate, 0.1 ml of KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (125, 250, 500 and 1000 µg/ml) of plant extracts in a final volume of 1.0 ml. The mixture was incubated at 37 °C for 1h. At the end of the incubation

period, 1ml of TBA was added and heated at 95 °C for 20 min to develop the colour. After cooling, the TBA formation was measured spectrophotometrically (Hitachi U-20) at 532 nm against an appropriate blank. The hydroxyl radical scavenging activities were determined by comparing the absorbance of the control with samples. The per cent TBA production for positive control vitamin C was fixed at 100% and the relative per cent TBA was calculated for the extracts.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 2.8 Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activities of the leaf samples were evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [17]. Aliquots of 0.4 mL of sample solution were combined in a vial with 4 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium

phosphate and 4Mm ammonium molybdate). The vials were capped and incubated in a water bath at 95 °C for 90 min. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activities were expressed relative to that of Vitamin C.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 2.9 Ferric reducing antioxidant power

The Ferric reducing antioxidant potential of various crude extracts of leaf sample as per the method of Oyaizu [18]. The samples were mixed with 2.5 ml of 0.2 M Phosphate buffer (pH 6.6) and 2.5 ml of 1 per cent potassium ferric cyanide. After the mixture was incubated at 50 °C for 20 min, 2.5 ml of 10 per cent TCA, 2.5 ml distilled water and 0.5 ml of 0.1 per cent ferric chloride was added and then the absorbance was measured at 700 nm against a blank. The blank consist of all the reagents without the test sample. The reducing power of Gallic acid was also determined for a comparison. High absorbance of the reaction mixture indicates strong ferric reducing antioxidant power.

### 2.9.1 Total phenol content

Total phenolic content was carried out following the Folin-Ciocalteu method described by Singleton and Rossi [19]. One ml of crude leaf extracts solution containing (1mg /ml) was added volumetric flask. 1 ml of Folin-Ciocalteu reagent and allowed to stand at 22 °C for 5 min; 7.5% of 0.75 ml of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured

spectrophotometrically (Hitachi U-20) at 765 nm using Spectrometer after 90 min at 22 °C. The amount of total phenolic content was determined as Gallic acid and equivalent and expressed as mg GAE/g.

### 2.9.2 Total flavonoid content

The flavonoids content was determined by aluminum trichloride method using catechin as a reference compound [20]. This method based on the formation of a complex flavonoid-aluminum having the absorptive spectrophotometrically (Hitachi U-20) maximum at 415 nm, after remained react at room temperature for 30 min. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The total flavonoid content was determined as mg QE/g.

### 2.9.3 Statistical analysis

The results are expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test

was performed to determine any significant difference between different extracts for *in vitro* antioxidant activity assays. Comparison of means for *in vivo* antioxidant activity assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. *P* value < 0.05 was considered statistically significant.

### 3. Results And Discussion

#### 3.1 DPPH (1,1- diphenyl-2-picrylhydrazyl hydrate radical scavenging activity)

The petroleum ether, chloroform, ethyl acetate and methanol leaves extracts of *P. dicoccos* exhibited the highest DPPH activity. The methanol extracts of *P. dicoccos* leaf, and Vitamin C (standard) IC<sub>50</sub> values were ranged from 184.35 and 156.76 µg/mL (Fig. 1). Wansi *et al.* [21] determined the radical scavenging activity of methanol extract of the stem bark of *Klainedoxa gabonensis* using 1,1-

diphenylpicrylhydrazyl (DPPH) assay gave high antioxidant values for the methanol extract (IC<sub>50</sub>) = 10.45 µg/mL. Ethanol and chloroform extracts of *Asteracantha longifolia* have more scavenging effect on DPPH radical than the standard having IC<sub>50</sub> value of 32.48 ± 0.58 and 30.47 ± 0.96 µg/mL respectively [22]. Shyura *et al.* [23] studied the scavenging DPPH radical activity of *Ludwigia octovalvis*, *Vitis thunbergii*, *Rubus parvifolius*, *Lindernia anagallis* and *Zanthoxylum nitidum* and their IC<sub>50</sub> values were 4.6, 24, 27, 36, 50 µg/mL respectively.

#### 3.2 Abts<sup>+</sup> Scavenging Effects (2, 2'-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid)

The different leaf extracts of *P. dicoccos* exhibited the highest ABTS<sup>+</sup> activity. The IC<sub>50</sub> values of methanol extracts of *P. dicoccos* leaves, and Vitamin C (standard) values were ranged from 193.29 and 168.46 µg/mL (Fig. 2).

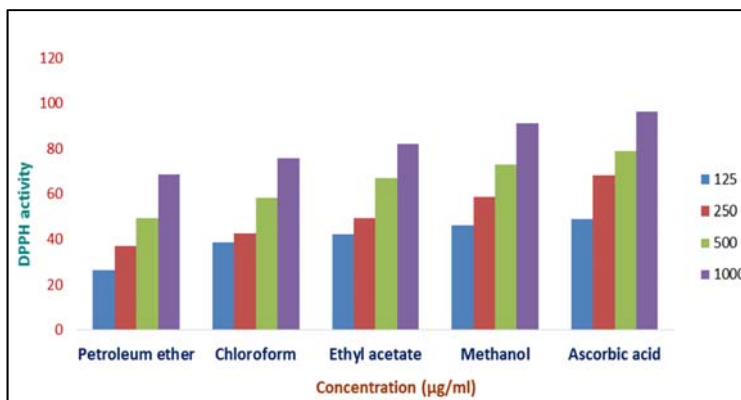


Fig 1: DPPH activity of different extracts of *Psudrax dicoccos* leaves

Jamuna *et al.* [24] reported that the chloroform and ethyl acetate extracts of root part of *Hypochaeris radicata* exhibited higher ABTS<sup>+</sup> radical scavenging activity. On the other hand, in our results the ABTS<sup>+</sup> activity of leaf, root and stem extracts of *Acalypha indica* was shown in the order of acetone > methanol > ethyl acetate > chloroform > petroleum ether. Miliuskas *et al.* [25] found that the methanol extract of 12 medicinal aromatic plants have investigated for their radical scavenging activity using DPPH and ABTS<sup>+</sup> assays: *Salvia sclarea*, *salvia glutinosa*, *Salvia pratensis*, *Lavandula angustifolia*, *Calendula officinalis*, *Matricaria recutita*, *Echinacea purpurea*, *Rhaponticum carthamoides*, *Juglans regia*, *Melilotus*

*officinalis* *Geranium macrorrhizum* and *Potentilla fruticosa*, *Salvia officinalis* was used as a reference plant with well documented antioxidant activity. The extracts of all the tested *salvia* species possessed high radical scavenging abilities.

Senthilkumar and Venkatesalu [26] studied the antioxidant and antimicrobial activities of fruit pulp essential oil of wood apple, *Feronia limonia*, for its chemical constituents. The antioxidant activity showed that the essential oil had good scavenging potential *viz.* DPPH radical (IC<sub>50</sub> = 41.35 g/mL), H<sub>2</sub>O<sub>2</sub> (IC<sub>50</sub> = 45.49 g/mL), O<sub>2</sub> (IC<sub>50</sub> = 30.86 g/mL), OH (IC<sub>50</sub> = 25.05 g/mL) and ABTS (IC<sub>50</sub> = 30.28 g/mL).

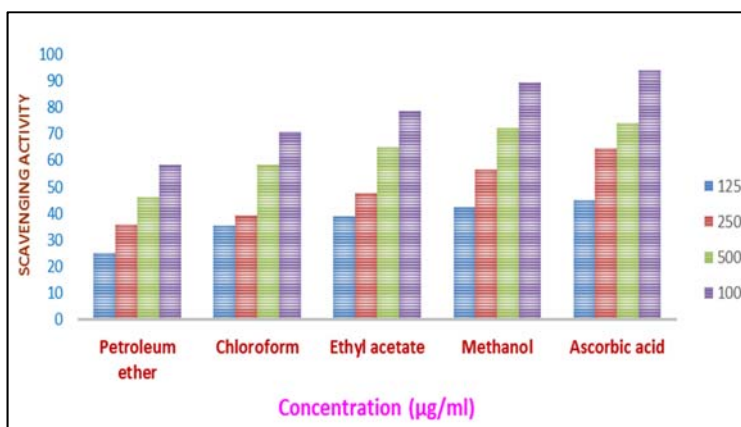


Fig 2: ABTS<sup>+</sup> scavenging effects (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) different extracts of *Psudrax dicoccos* leaf

### 3.3 Hydrogen Peroxide Scavenging effects

The petroleum ether, chloroform, ethyl acetate and methanol leaf extracts of *P. dicoccos* exhibited the highest H<sub>2</sub>O<sub>2</sub> activity. The IC<sub>50</sub> values of methanol extracts of *P. dicoccos* leaves, and Vitamin C (standard) values were ranged from 467.48 and 324.40 µg/mL (Fig. 3). Dhruvi [27] reported that the antioxidant activity of the methanolic and aqueous extracts of *Martynia annua* leaves. The antioxidant property

depends upon concentration and it increased with increasing amount of the extract. The free radical scavenging and antioxidant activity may be attributed to the presence of phenolic and flavonoid compounds present in the extract. The results showed that the ethyl acetate extract exhibited higher antioxidant activity than the methanol, chloroform and hexane extracts.

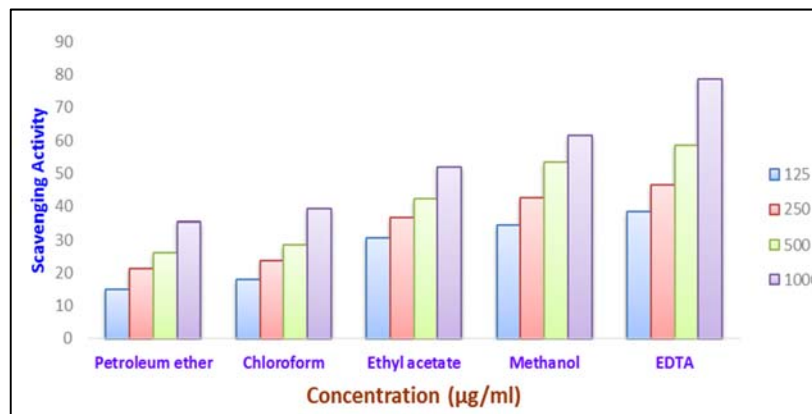


Fig 3: Hydrogen peroxide scavenging effects of different extracts of *Psyrdrax dicoccos* leaf

### 3.4 Superoxide anion scavenging activity

The different leaf extracts of *P. dicoccos* exhibited the highest superoxide anion activity. The IC<sub>50</sub> values of methanol extracts of *P. dicoccos* leaf, and Vitamin C (standard) values were ranged from 534.167 and 512.48 µg/mL (Fig 4.). The superoxide anion scavenging activity was observed in plant extracts of *Ludwigia octovalvis* (26 µg/mL), *Vitis thunbergii* (58 µg/mL), *Prunella vulgaris* (113 µg/mL), *Saurauia oldhamii* (124 µg/mL) and *Rubus parvifolius* (151 µg/mL). The highest phenol content 2218 mg/L (mg CE/L) was found in *Melissae folium* [28].

Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents, e.g. hydroxyl radical [29]. Antioxidant activity of methanolic extract of *Abutilon indicum* leaves were investigated for its free radical scavenging activity and the maximum scavenging of nitric oxide radical found were 28.74% and 49.62% respectively at 250 µg/ml concentration [30].

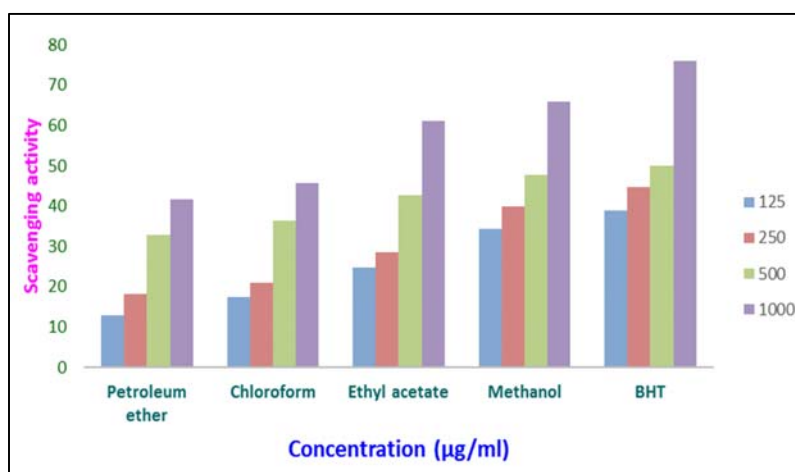
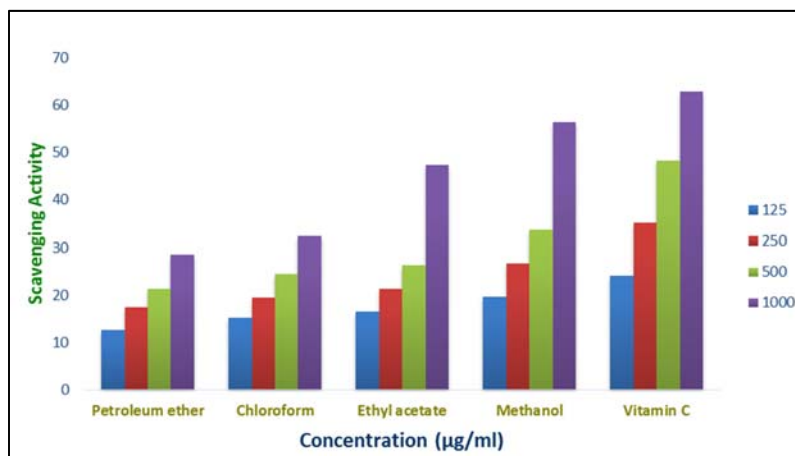


Fig 4: Superoxide radical scavenging activity of different extracts of *Psyrdrax dicoccos* leaf

### 3.5 Hydroxyl radical scavenging activity

The petroleum ether, chloroform, ethyl acetate and methanol leaves extracts of *P. dicoccos* exhibited the highest hydroxyl radical activity. The IC<sub>50</sub> values of methanol extracts of *P. dicoccos* leaf, and Vitamin C (standard) values were ranged from 467.148 and 324.40 µg/mL. The IC<sub>50</sub> values of ethyl acetate extract of *P. dicoccos* leaf, and Vitamin C values were ranged from 984.78 and 324.40 µg/mL. The IC<sub>50</sub>

values of chloroform extract of *Psyrdrax dicoccos* leaf, and Vitamin C values were ranged from 1532.56 and 324.40 µg/mL. The IC<sub>50</sub> values of petroleum ether extract of *P. dicoccos* leaf, and Vitamin C values were ranged from 1754.34 and 324.40 µg/mL (Fig. 5).



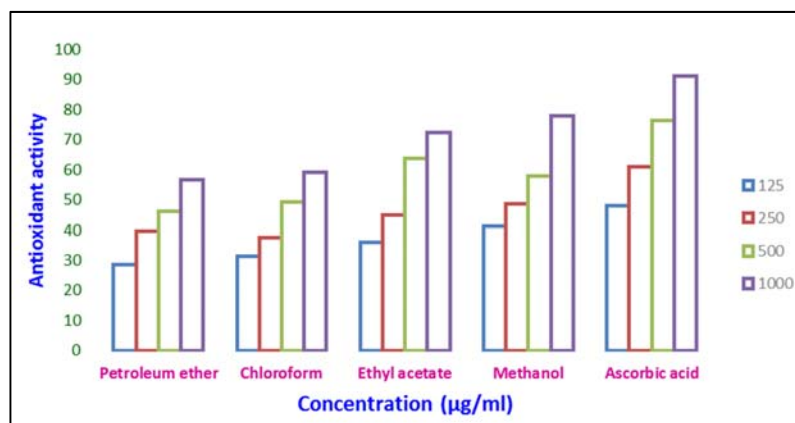
**Fig 5:** Hydroxyl radical scavenging effects of different extracts of *Psyrdrax dicoccos* leaf

Hydroxyl radical is highly reactive oxygen, centered radical, formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid membranes and most biological molecule it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reaction of lipids<sup>[31]</sup>

### 3.6 Total antioxidant activity

The petroleum ether, chloroform, ethyl acetate and methanol leaves extracts of *P. dicoccos* exhibited the highest total

antioxidant. The IC<sub>50</sub> values of methanol extracts of *P. dicoccos* leaves, and Vitamin C (standard) values were ranged from 272.136 and 154.43 µg/mL. The IC<sub>50</sub> values of ethyl acetate extract of *P. dicoccos* leaves, and Vitamin C values were ranged from 298.49 and 154.43 µg/mL. The IC<sub>50</sub> values of chloroform extract of *P. dicoccos* leaves, and Vitamin C values were ranged from 516.32 and 154.43 µg/mL. The IC<sub>50</sub> values of petroleum ether extract of *P. dicoccos* leaves, and Vitamin C values were ranged from 567.49 and 154.43 µg/mL (Fig.6)



**Fig 6:** Total antioxidant scavenging effects of different extracts of *Psyrdrax dicoccos* leaf

Ashafa *et al.*<sup>[32]</sup> have reported total antioxidant activity of the acetone extracts of *Felicia muricata*. It was found to be a good total antioxidant activity due to the presence of gallic acid. The antioxidant activity of methanolic extracts from the leaves and stem of *Mollugo nudicaulis* the presence of significant quantities of total phenolics, flavonoids<sup>[33]</sup>.

### 3.7 Ferric reducing antioxidant power assay

The different leaves extracts of *P. dicoccos* exhibited the highest ferric reducing antioxidant power. The IC<sub>50</sub> values

of methanol extract of *P. dicoccos* leaf, and Vitamin C (standard) values were ranged from 486.46 and 284.57, µg/mL. The IC<sub>50</sub> values of ethyl acetate extract of *P. dicoccos* leaf, and Vitamin C values were ranged from 636.89 and 284.57 µg/mL. The IC<sub>50</sub> values of chloroform extract of *P. dicoccos* leaf, and Vitamin C values were ranged from 975.42 and 284.57 µg/mL. The IC<sub>50</sub> values of petroleum ether extract of *P. dicoccos* leaves, and Vitamin C values were ranged from 1056.48 and 284.57 µg/mL (Fig.7)

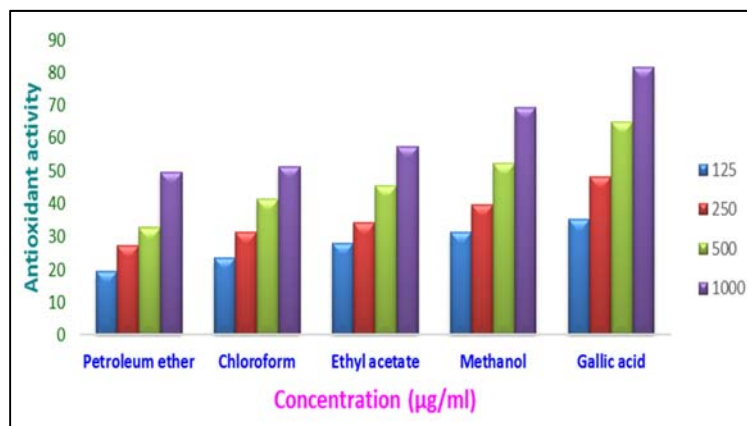


Fig 7: Ferric Reducing Antioxidant Power Assay in different extracts of *Psyrax dicoccos* leaf

The ferric reducing antioxidant power is increased with increasing concentrations in all the samples but the acetone extract of leaves exhibited the significant effect in comparison with other solvent extracts. These findings supported the investigation of Ruan *et al.* [34] on *Syzygium cumini* leaf extracts.

Ahmad and Khan [35] suggested that the antioxidant activity of leaf extracts of *Abutilon indicum* was evaluated to explore new bioactive compatibles with least associated side effects. The methanol extracts was prepared and screened for *in vitro* by using FRAP. The reducing power of methanolic leaf extract was markedly increase by increasing concentration. The results indicated a strong antioxidant activity.

### 3.8 Total phenol and flavonoid content

Total phenol and flavonoid contents were extracted by organic solvents with different polarities (petroleum ether, chloroform, ethyl acetate and methanol). The results are presented in Table 4.2.6, showed the differences in the total phenolic and flavonoid content in different parts of *P. dicoccos*. The total phenol ( $6.38 \pm 0.76$ ) mg gallic acid equivalent (GAE/g) and flavonoids ( $8.18 \pm 0.37$ ) mg quercetin equivalent (CE/g) were found to be higher in methanol extract of leaves of *P. dicoccos* (Table 1&2).

Table 1: Total phenol content in different extracts of *Pleiospermium alatum* leaves

Extracts	<i>Pleiospermium alatum</i> Leaves	
	Total phenol GAE (50µg/ml)	Total flavonoid QE (50µg/ml)
Petroleum ether	$1.13 \pm 0.10^a$	$2.12 \pm 0.46^a$
Chloroform	$1.47 \pm 0.76^a$	$2.50 \pm 0.08^a$
Ethyl acetate	$3.59 \pm 0.50^{ab}$	$5.46 \pm 0.68^{ab}$
Methanol	$5.05 \pm 0.76^b$	$6.72 \pm 0.13^b$
Gallic acid/Quercetin	$7.51 \pm 0.50^c$	$9.36 \pm 0.76^c$

<sup>a</sup>-mean of three assays;  $\pm$  - standard deviation; \*\* significant at  $p < 0.05$

Leandro *et al.* [36] studied the total phenol and flavonoid contents of extracts from the plant leaf, bark, root, fruit and/or stem of 19 Amazonian plants and their related antioxidant activities. Total phenols ranged from 0.8 to 22.2 mg gallic acid equivalents/g and flavonoids from 0.0 to 10.2 mg catechin equivalents/g. All the extracts showed different degrees of antioxidant activity with TEAC = 1.1 up to 117.4 and ORAC = 7.8 up to 359.1  $\mu$ mol Trolox equivalents/g.

*Piper putumayoense*, *P. glandulosissimum*, *P. krukoffii* and *Senna reticulata* leaves and *Brownea rosademonte* bark showed elevated antioxidant activities. Katalinic *et al.* [28] investigated the total phenolic content and related total antioxidant capacity of 70 medicinal plant infusions. The total phenolic content of medicinal plant infusions ranges from 9 to 2218 mg/L. The FRAP range from 0.06 to 25 mM/L. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [37]. The concentration of flavonoids in the extracts depends on the polarity of solvents and the type of plant material used for the extractions [38]. Flavonoids have been reported to possess many useful properties, including antimicrobial, anti-inflammatory, antioxidant, antiallergic, hepatoprotective, anti-thrombic, antiviral and anti-carcinogenic activities [39].

The higher amount of phenol and flavonoids content were present in the acetone extracts of leaves than that of other parts of solvent extracts. The phenolic compounds are known as powerful chain breaking antioxidant. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl group and may contribute directly to antioxidative action [40]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [41].

### 4. Conclusion

From the study it is concluded that the antioxidant capacities, total phenolic and flavonoid contents of the leaves which are widely used by the India are considered as good sources of antioxidants as observed in DPPH scavenging assay. Among *Psyrax dicoccos* has the highest antioxidant activity antioxidants with consequent health benefits particularly *Psyrax dicoccos* can be consider as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetics aging and cardiovascular diseases.

### 5. Acknowledgement

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