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Phytochemical profiling and antioxidant activity of the extracts of *Pachygone ovata* (Poir.) Miers ex Hook.f. & Thomson

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Abstract

The current study was carried out to assess the *in vitro* antioxidant properties of various extracts of stem and leaf parts of *Pachygone ovata* and to analyze its secondary metabolites. Hexane, petroleum ether, chloroform and ethanol were used as extraction solvents and are employed for phytochemical screening. Antioxidant assays were evaluated by assessing DPPH radical scavenging assay, nitric oxide radical inhibition activity and reducing power activity. Phytochemical analysis of the extracts of *P. ovata* indicated the presence of various types of compounds. Among the tested solvent extracts, hexane and chloroform reported maximum yield. In *in vitro* antioxidant activity, chloroform extract of stem, on DPPH and Nitric Oxide showed a greater scavenging effect as well as high reducing potential when compared to other solvent extracts. From this study, it can be concluded that the secondary metabolites and antioxidant nature of the compounds present in the plant material can be used to invent new drugs of antioxidant properties of natural origin.

Keywords: *P. ovata*, DPPH activity, phytochemical screening, antioxidant, *in vitro*

Introduction

Plant-derived substances have become one of the major products in pharma industries owing to their versatile applications. Hence many medicinal plants and herbs are explored and tested for their biomedical properties. Studies on phytochemical analyses reveal the chemical nature of the plants. These natural products and its bioactive compounds represent a promising direction in phytopharmacy. Due to the modernization, the treasure of traditional medicinal knowledge is gradually declining ^[1]. Therefore researchers are focusing their interests on medicinal plants to evaluate its antioxidant and phytochemical properties in their potential role for preventing various human diseases ^[2]. Pharmacognostical evaluation provides diagnostical characters which would be useful in identification and authentication of the drug in the herbal industry. The conventional use of synthetic antioxidants is succeeded by the natural oxidants since it is considered as safer without any side effects ^[3].

Based on the literatures, the species of the Menispermaceae family have been used in Indian folk medicine for the treatment of diabetes, oedema, pain, rheumatoid arthritis, bone fracture, nephritis, pyrexia and hypertension ^[4-7]. *Pachygone ovata* has been an unexplored and trivialized plant ^[8] that belongs to the Menispermaceae family. Genus of *P. ovata* is found in southern parts of India like Tamil Nadu, Karnataka and Andhra Pradesh ^[9]. The ancient folks considered this whole plant to be medicinally important, since the dried fruit was used as fish poison and vermicide; the leaves were used to reduce body temperature and improve fertility and it also possesses various pharmacological actions including Analgesic, CNS stimulant and to cure hypothermia & leucorrhea. There has not been much scientific data available that justifies the pharmacological attributes of *P. ovata*. Hence the present investigation was performed to screen the phytochemical action and antioxidant ability of the plant.

Materials and Methods

Source of Collection

Fresh leaves and stem of *Pachygone ovata* were collected from Tirunelveli District of Tamil Nadu, India and was verified by Dr. V. Chelladurai, Research officer – Botany (Scientist C), Central Council for Research in Ayurveda and Siddha, Government of India. The herbarium voucher number is LCH 407. The samples were dried completely at room temperature under shade. Dried materials were ground into coarse powder and stored in airtight container for further works.

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Plant extract preparation

Fresh leaves and stem were collected, shade dried and powdered. The powder (150 gm) was extracted three times by cold percolation method with 450 ml of hexane, petroleum ether, chloroform and ethanol for 72 h. The filtrates were concentrated under reduced pressure at 40 °C and stored in refrigerator at 2-8 °C for further use in subsequent experiments. The yield of the extracts was noted and percentage yield was calculated as per the below formula:

$$\text{Yield (\%)} = \frac{W_2 - W_1}{W_0} \times 100$$

Where: W₂ = weight of the extract and the container,
W₁ = weight of the container alone,
W₀ = weight of the initial dried sample

Phytochemical Screening

The crude powder of *P. ovata* was subjected to phytochemical analysis to determine the presence/absence of different phytoconstituents using standard protocols of Harborne *et al* (1973)^[10].

Test for Carbohydrates (Molisch's Test)

1 ml of Molisch's reagent was added to 2 ml of plant extract and a few drops of concentrated sulfuric acid were added. Formation of purple or reddish color indicated the presence of carbohydrates.

Test for Tannins

1 ml of plant extract was added to 2 ml of 5% ferric chloride solution. Formation of dark blue or greenish black coloration indicated the presence of tannins.

Test for Saponins (Foam Test)

2 ml of distilled water was added to 2 ml of plant extract and shaken in a graduated cylinder for 15 min lengthwise. The formation of 1 cm layer of foam indicated the presence of saponins.

Test for Flavonoids (Alkaline Reagent Test)

1 ml of 2N sodium hydroxide was added to 2 ml of plant extract. Formation of yellow color was observed, indicating a positive test for flavonoids.

Test for Alkaloids (Meyer's Test)

2 ml of concentrated hydrochloric acid was added to 2 ml of plant extract followed by a few drops of Mayer's reagent. Formation of green color or white precipitate indicated the presence of alkaloids.

Test for Quinones

1 ml of concentrated sulphuric acid was added to 1 ml of plant extract. Formation of red colour indicated the presence of Quinones.

Test for Glycosides

3 ml of chloroform and 10% ammonia solution was added to 2 ml of plant extract. Appearance of pink color indicated the presence of glycosides.

Test for Cardiac Glycosides (Keller Killiani Test)

2 ml of glacial acetic acid was added to 0.5 ml of extract and few drops of 5% ferric chloride solution were added. This was under layered with 1 ml of concentrated sulphuric acid. The

formation of brown ring at the interface indicated the presence of cardiac glycosides.

Test for Terpenoids (Salkowski Method)

2 ml of chloroform was added to 0.5 ml of extract, followed by adding concentrated sulphuric acid. The appearance of red brown colour of the interface indicates presence of terpenoids.

Test for Phenols (Ferric Chloride Test)

1 ml of the extract was mixed with 2 ml of distilled water, followed by a few drops of 10% ferric chloride. Formation of blue or green coloration indicated the presence of phenols.

Test for Coumarins

1 ml of 10% NaOH was added to 1 ml of extract and formation of yellow color was observed indicating the presence of coumarins.

Test for Steroids and Phytosteroids

Equal volume of chloroform was added to 1 ml of plant extract and subjected with few drops of concentrated sulphuric acid. Formation of brown ring indicated the presence of steroids and bluish brown ring indicated the presence of phytosteroids.

Test for Protein (Ninhydrin Test)

1 ml of Ninhydrin solution was added to 1 ml of sample extract, the formation of blue color was noticed.

Test for Phlobatannins

Few drops of 2% hydrochloric acid were added to 1 ml of plant extract. Appearance of red color precipitate indicated the presence of phlobatannins.

Test for Anthraquinones (Borntrger's Test)

10% ammonia solution was added to 1 ml of plant extract. Formation of pink coloration in the precipitate indicated the presence of anthraquinones.

In vitro antioxidant activities

The antioxidant activity of the various solvent extracts of the plant was determined by employing the following methods.

DPPH radical scavenging assay

The free radical scavenging by DPPH was performed, described by Blois^[11]. A stock solution of plant extracts was prepared to the concentration of 10 mg/ml. Different concentrations of the extract (200, 400, 600, 800 and 1000 µg/ml) were added at an equal volume to the methanolic solution of DPPH (0.1 mM). The reaction mixture was incubated for 30 min at room temperature; the absorbance was recorded at 517 nm. The experiment was done in triplicate. Ascorbic acid was used as a standard control. The annihilation activity of free radicals was calculated in % inhibition according to the following formula

$$\% \text{ of Inhibition} = \frac{(A \text{ of control} - A \text{ of Test})}{A \text{ of control}} \times 100$$

The antiradical activity of the extract was expressed as IC₅₀ (µg/ml) which is half maximal inhibitory concentration and is defined as the microgram of the extract to scavenge the DPPH radicals by 50%.

Nitric oxide radical inhibition activity

Nitric oxide scavenging activity can be estimated by the use of Griess Illosvoy reaction [12]. In the present study Griess Illosvoy reagent was modified using naphthylethylene diaminedi hydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentrations of extract (200– 1000 µg/ml) or standard solution (0.5 ml) were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diaminedihydrochloride (1%) was added, mixed and allowed to stand for 30 min. A pink colored chromophore was formed in diffused light. The absorbance of the solution was measured at 540 nm against the corresponding blank. Vitamin C was used as positive control. The scavenging activity was calculated using the formula,

$$\% \text{ of Inhibition} = (A \text{ of control} - A \text{ of Test}) / A \text{ of control} * 100$$

Reducing power activity

A modified method [13] was adopted for the ferric reducing antioxidant power (FRAP) assay. The different concentrations of standard or sample extract (200, 400, 600, 800 and 1000 µg/ml) was mixed with 300 µl of ferric-TPTZ reagent [prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM FeCl₃·6H₂O at a ratio of 10:1:1 (v/v/v)]. The mixture was incubated at 37 °C, and the absorbance readings were taken at 593 nm after 4 min. Results were expressed in mM Fe(II)/g dry mass using a

calibration curve of a freshly prepared ferrous sulfate solution.

$$\text{FRAP value} : [A (\text{Sample Final}) - A (\text{Sample Initial}) / A (\text{Std Final}) - A (\text{Std Initial})] \times 2$$

Statistical analysis

The experiments were carried out in triplicates and the data has been expressed as Mean ± SD. For deriving IC₅₀ values and identifying the significance of the difference between means ($p < 0.05$) from Student's t test, a statistical tool Graph pad Prism Software version 7.01, was used.

Results

Quantitative analysis of *P. ovata* extract yield

The yield of the hexane, petroleum ether, chloroform and ethanol extracts were 7.372 g, 0.438 g, 2.244 g and 0.321 g in leaf and 0.1935 g, 0.027g, 2.454g, and 0.280g in stem respectively. Percentage yield was calculated and found to be 4.91%, 0.29%, 1.49% and 0.21% in leaf and 0.12%, 0.01%, 1.63% and 0.18% in stem respectively. Highest percentage yield was obtained in leaf with hexane and in stem with chloroform. Lowest percentage yield was obtained in leaf with ethanol and in stem with petroleum ether.

Preliminary qualitative analysis of phytochemical tests

The present study revealed that the solvent extracts of leaf and stem of *P. ovata* contained high number of carbohydrates, tannins, alkaloids and phenols, moderate number of quinones, cardiac glycosides. Only traces of steroids and saponins were noted, while the presence of flavonoids, terpenoids and coumarins were significantly less. Protein, phlobatannins, anthraquinones and glycosides gave negative results on both stem and leaf extracts. The results have been shown in table 1.

Table 1: Qualitative Phytochemical analysis of *P. ovata* with different solvent extracts

Name of the Test	Name of the Sample							
	Stem				Leaf			
	Hexane	Pet. Ether	Chloroform	Ethanol	Hexane	Pet. Ether	Chloroform	Ethanol
Carbohydrates	++	++	++	++	++	++	++	++
Tannins	++	++	++	++	++	-	++	++
Saponins	-	-	-	-	-	+	-	-
Flavonoids	-	-	-	-	-	+	-	++
Alkaloids	+	+	++	++	+	-	++	++
Quinones	+	++	++	+	-	-	-	-
Glycoside	-	-	-	-	-	-	-	-
Cardiac glycosides	++	++	-	++	++	++	-	++
Terpenoids	-	-	-	-	++	-	+	+
Phenols	++	+	++	++	-	++	++	++
Coumarins	+	-	-	-	-	++	-	+
Steroids & Phytosteroids	++	+	-	-	-	-	-	-
Proteins	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-

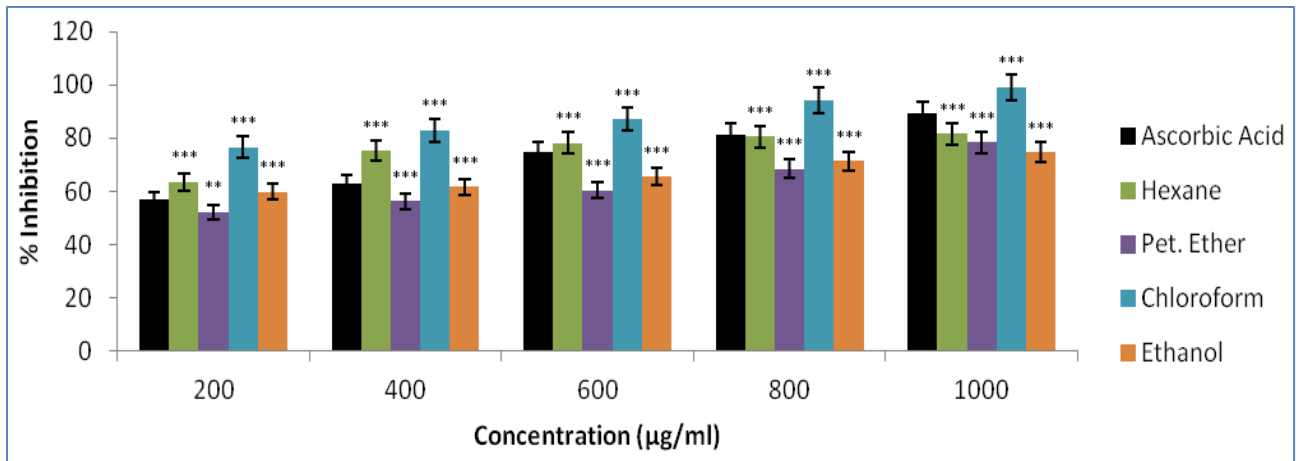
(++) = Highly Present, (+) = Present, (-) = Absent

Antioxidant assays of various solvent extracts of *P. ovata*

DPPH radical scavenging activity

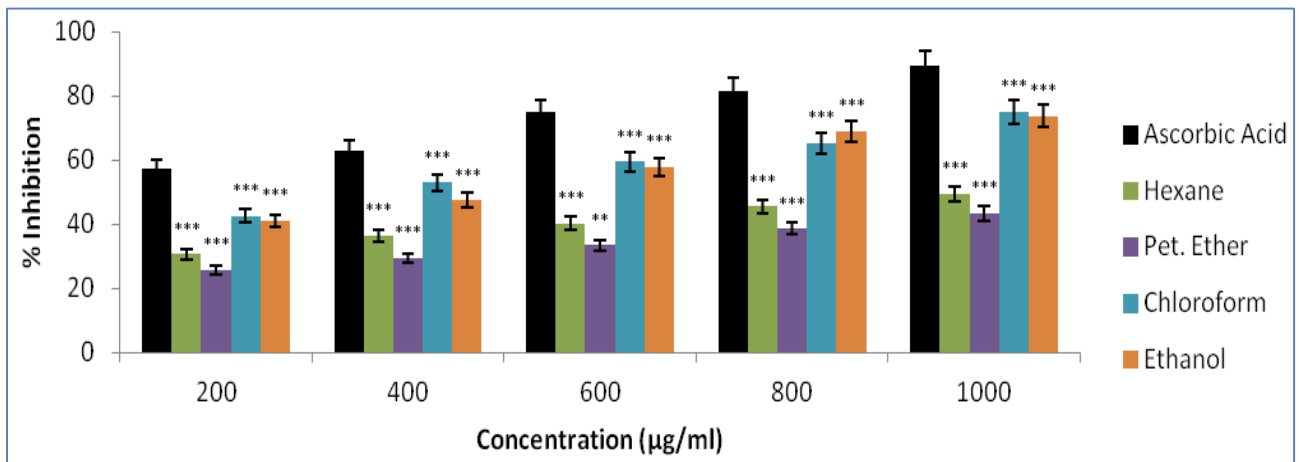
In the present study, the solvent extracts had significant scavenging effects on the DPPH radical which was increasing with increase in the concentration of the sample from 200-1000 µg/ml. The 50% inhibition of DPPH radical (IC₅₀) of the plant extracts were determined; a lower value would reflect greater antioxidant activity of the sample. It was found that the radical scavenging activity of *P. ovata* was highly

effective for the chloroform extract of both stem and leaf (69.25 and 324 µg/ml respectively). The IC₅₀ values for the other extracts of the stem were found to be 75.35 µg/ml, 90.97 µg/ml and 215.3 µg/ml for the hexane, ethanol and petroleum ether, respectively. Likewise, the IC₅₀ value for the ethanol extract of the leaf was 362.1 µg/ml whereas Hexane and Petroleum Ether extracts resulted in higher IC₅₀ values, exhibiting poor scavenging activity (Fig. 1 & 2). The IC₅₀ value of ascorbic acid was 171 µg/ml.



* represents the level of significance (** $p < 0.01$, *** $p < 0.001$)

Fig 1: DPPH activity of *P. ovata* stem extracted in different solvents



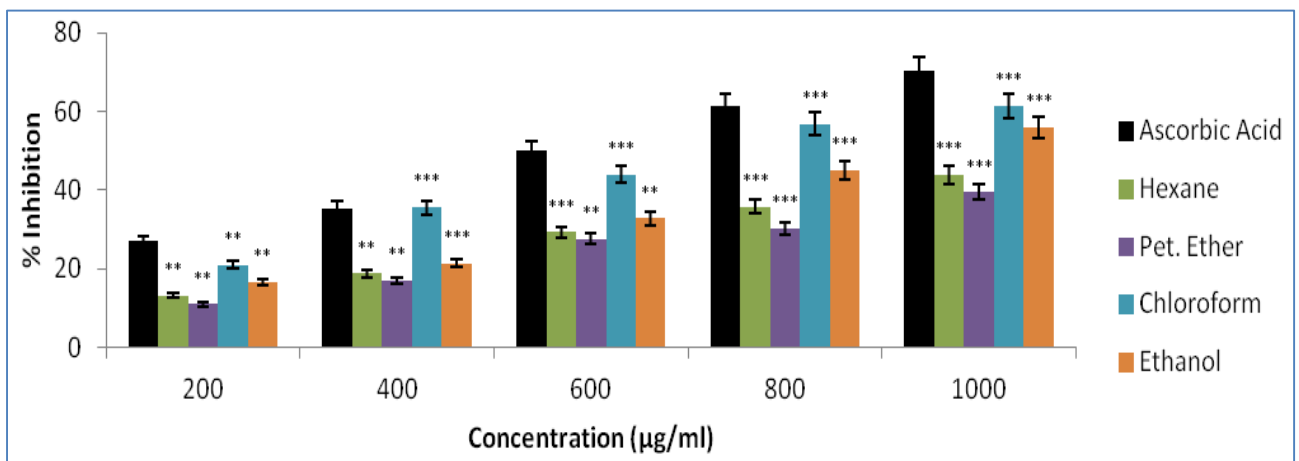
* represents the level of significance (** $p < 0.01$, *** $p < 0.001$)

Fig 2: DPPH activity of *P. ovata* leaf extracted in different solvents

Nitric oxide radical scavenging activity

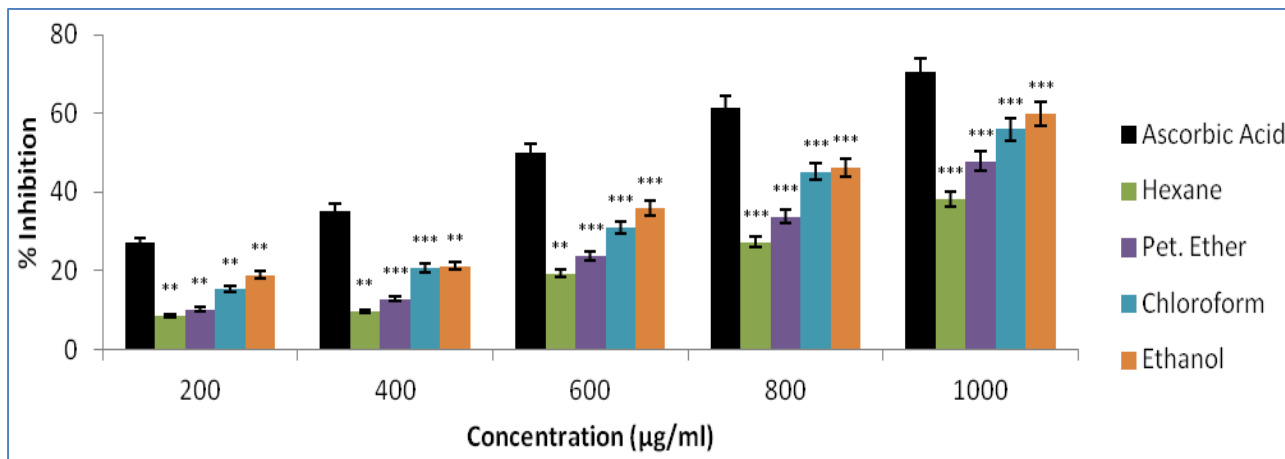
The scavenging of nitric oxide activity exhibited strong IC₅₀ value for the chloroform extract of stem(671.8 µg/ml) and the antioxidant activity was decreased in the order of Chloroform>Ethanol>Hexane>Petroleum Ether. Similarly,

the IC₅₀ value for the ethanol extract of leaf was 852.5 µg/ml and the other solvent extracts showed lesser antiradical activity when compared to ethanol extracts (Fig. 3 & 4). The IC₅₀ value of ascorbic acid was 557.7 µg/ml.



* represents the level of significance (** $p < 0.01$, *** $p < 0.001$)

Fig 3: Nitric oxide scavenging activity of *P. ovata* extracts (Stem)



* represents the level of significance (** $p < 0.01$, *** $p < 0.001$)

Fig 4: Nitric oxide scavenging activity of *P. ovata* extracts (Leaf)

Reducing power activity

Concentration dependency of antioxidant potential was investigated as a function of reducing power. The reductive capabilities of Hexane, Petroleum Ether, Chloroform and Ethanol extracts of stem and leaf of *P. ovata* were compared with Ascorbic acid. A higher absorbance value indicates a

stronger reducing power of the samples, as shown in Fig. 5 & 6. The reducing power of Petroleum Ether and Chloroform extract of stem was very potent than Hexane and Ethanol extracts. Similarly, the Chloroform extract of leaf showed highest reducing power activity compared to other solvents.

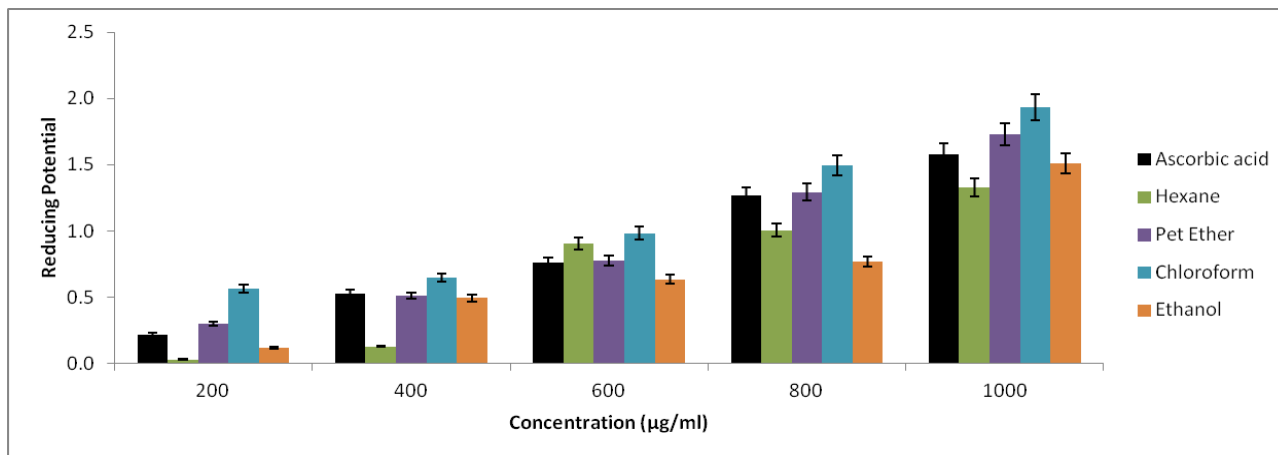


Fig 5: FRAP assay of *P. ovata* extract (Stem)

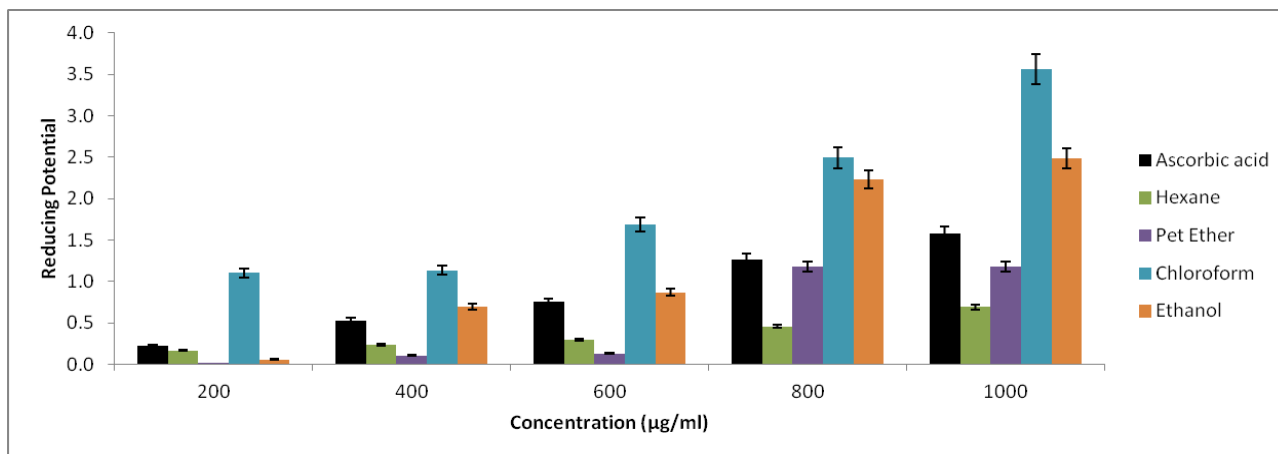


Fig 6: FRAP assay of *P. ovata* extract (Leaf)

Discussion

As reported by [14] alkaloids, tannins, flavonoids and phenolic compounds are considered to be the most important bioactive ingredients of plants. The plant extract in our study showed high carbohydrates, tannins, alkaloids and phenols. Among

the various solvent extracts of medicinal plants tested, hexane and chloroform extracts had maximum yield of phytochemicals. High yield of hexane extract of leaf and chloroform extract of stem indicates the presence of high concentrations of essential oils and other low polar

phytochemicals such as tannins and phenols. Low yield of ethanolic extracts in both leaf and stem may be because of the leaching of low polar phytochemicals in hexane and chloroform. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites^[15].

In vitro antioxidant assay in *P. ovata* showed that the DPPH reduction took place with increase in concentration of the sample indicating that samples had antioxidant property. Samples showed excellent antioxidant properties in chloroform extract of stem. Tannins exhibit stringent properties that can speed up healing process and inflamed mucous membranes^[16]. Phenols show high solubility in polar solvents and our results are also in accordance with^[17] samples showed maximum number of positive result on the presence of tannins and phenols which could be one of the reasons for high scavenging activity. These phenolic compounds embrace a wide range of plant substances and are very important plant metabolites^[18] and considered to be principal antioxidant compounds in many plant species like fruits, vegetables and medicinal plants^[19-21].

The decrease in the formation of purple azo dye reflects the presence of scavengers in the test compounds^[22]. It clearly indicates that this plant extract has a noticeable effect as scavenging nitric oxide radicals. Reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity^[23]. The reducing power increased with the increase in the extract concentrations. Hence, this study presumed that *P. ovata* may have a high amount of reductones in the extracts.

Conclusion

This phytochemical and antioxidant study helped in concluding the nature of the compounds and secondary metabolites present in the plant material. It also signifies that *Pachygone ovata* can be investigated as a prospective source for new drugs in treating various dreadful diseases provided advance research work is carried out for isolation and identification of the bioactive compound from this plant.

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