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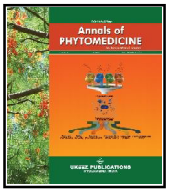

Original Article : Open Access

Preliminary phytochemical analysis and antioxidant activity of leaf, stem and callus extracts of *Poeciloneuron indicum* Bedd.: An endemic medicinal plant of Western Ghats

Manasa Sangeetha and Shobha Jagannath◆

Plant Tissue Culture Lab, DOS in Botany, University of Mysore, Mysuru-570006, Karnataka, India

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Abstract

Poeciloneuron indicum Bedd. is an endemic medicinal plant of Western Ghats belonging to the family Clusiaceae. Bark is used to treat dysentery, diarrhea and cholera and its root decoctionwas used as oral contraceptive. Phytochemical screening showed the presence of sterols, triterpenes, saponins, alkaloids, phenols, tannins, flavonoids, carbohydrates, resins, proteins and glycosides using extractsof hexane, chloroform, ethyl acetate, methanol and aqueous of stem, leaf and callus. Majority of were present in methanol extract and aqueous extract of leaf, whereas for stem in methanol extract and ethyl acetate extract and methanol extract of callus. Methanol extract of leaf, stem and callusshowed maximum phenols of 35.02 GAE/g, 32.05 GAE/g and 19.03 GAE/g, respectively, total flavonoid content wasmaximum with 628.57 mg/g in aqueous extract of stem followed by ethyl acetate extract of leaf with 585.71 mg/g and 342.62 mg/g in aqueous extract of callus extract. DPPH scavenging activity revealed highest scavenging activity of 89.53% inmethanol extract of stem and aqueous extract of leaf with 76.57%. Methanolic extracts of stem, leaf and callus have good reducing power with 0.461, 0.453 and 0.253 at 700 nm, respectively,the methanol extracts of stem, aqueous extract of leaf and methanolic extract of leaf derived callus extractsof *P. indicum* consists of active antioxidants.

1. Introduction

Phytochemicals are the major source of drugs since long back, which are produced by plants as primary and secondary metabolites in the various parts of the body and can be extracted by using many different techniques of modern era. Some of the major phytoconstituents such as phenols, terpenoides, saponins, flavonoids, sterols, triterpenes, alkaloids, tannins, resins, proteins, carbohydrates and glycosides will be present in different proportion in different species of the plant kingdom. Plants have been used tremendously in various fields of medicine. Plants of human-interest present on different regions of the earth, some have been proven to be effective and others need to be examined, tested and used in various fields of medicine. Antioxidants are not only produced in the plant body which is also produced in all living organisms, helping them in defense mechanism. But antioxidants in plant body are so high compared to the one with other living organisms that they can be derived and used for the purpose of drug and by consuming plant-based food containing adequate number of antioxidants helps to reduce many sever illness including cancer (Lobo*et al.*, 2010). This actually showcases the importance of plants in our lives both for healthy life and in the case of drug. Along with all these uses, antioxidants are used as food preservatives since they have the ability to increase mean-*life of food*. Phenolics are characterized to have anti-inflammatory, antioxidant, and anti-carcinogenic properties in common. Some act as antiseptic, anti-microbial and anthelmintic agents also. Some of the common phenolics includes phenol, hydroquinone, pyrogallol acid, salicylic acid, gallic acid, xanthones, flavonoids, phenol, etc., among which gallic acid and many of the flavonoids are known to have antioxidant property (Hano and Tungmunnithum, 2020).

Biodiversity hotspots are rich in *P. indicum* species, western Ghats region of India is one of the hotspots recognized and consists of the plant *Poeciloneuron indicum*, belongs to the family Clusiaceae also known as Guttiferae, is an important medicinal plant endemic to the regions of Western Ghats. Various parts have been used to treat different diseases like diarrhoea, dysentery, cholera and root extracts are used as an oral contraceptive (Shrisha *et al.*, 2011).

2. Materials and Methods

2.1 Callus induction

Callus powder used for the present study was obtained by using following method.

Leaf explants were prepared by tender green leaves collected from Kudremukh region. Leaves were washed for three to four times in running tap water and further surface sterilisation were made using 0.1 % of Mercuric chloride solution for less than 30 sec. These were then inoculated on sterilised MS media containing 1.5ppm TDZ inside laminar air flow. Media used was MS ready media purchased from Hi-media. Callus was collected once it attained maximum yield after 70 to 90 days of callus initiation and dried at 40°C for 48 h in hot air oven (Bano *et al.*, 2022). Dried callus were powdered and used for further phytochemical extraction.

Corresponding author: Dr. Shobha Jagannath

Plant Tissue Culture Lab, DOS in Botany, University of Mysore, Mysuru-570006, Karnataka, India

E-mail: shobhajags25@gmial.com

Tel.: +91-9448486596

2.2 Methods followed for phytochemical analysis

The plant materials necessary for carrying out the experiments were from Kuduremukh regionand identified with the help of Flora of Karnataka (Saldanha, 1984).The herbarium of plant material has been deposited in Herbarium of DOS in Botany, University of Mysore with accession number UOMBOT22P174. Leaves and stem were weighed, shade dried and powdered. All stem, leaf and callus samples were subjected for standard soxhlet extraction using different solvents.The extracts were used for phytochemical analysis which was evaluated according to standard procedure of Harborne (1973). Phytoconstituents present in different extracts are tabulated and compared in Table 1. After preliminary screening the extracts has been subjected to antioxidant activity assays.

2.3 Evaluation of antioxidants

2.3.1 Determination of phenolic content by FC method

1 ml of the respective extract to be tested for the concentration of polyphenols was dissolved in its respective solvent to get 1mg/ ml concentration of extract. 5 ml of the FC reagent was added. After incubation the mixtures were measured for their absorbance values at 760 nm against the above-mentioned blank solution. The same was repeated for varied concentration of standard solution and absorbance was recorded. The data obtained from standard are used for plotting calibration curve by taking concentration of gallic acid and absorbance on x and y axis, respectively, The concentrations of polyphenols in each extract were calculated using calibration curve and gallic acid equivalents were graphically plotted in Figures1, 2. Methods followed by Dudonne *et.al.*, (2009) and Stankovic *et al.*, (2011)were used for determining total phenolics present in different extracts.

2.3.2 Evaluation of flavonoid content

Procedure followed was of Chang *et al.,* (2002) with required changes. Method involves formation of complex between flavonoid and aluminium chloride which later be determined by spectro-photometric method since it provides bathochromic displacement and the hyperchromic effect (Lazon *et al.,* 2015).

Stock solutions of different extracts were prepared in 1 mg/ ml concentration of respective solvents from which 100 µl containing 100 µg was taken and diluted with methanol. 200µl of 10% Aluminium Chloride and 1 Molar Potassium acetate solutions followed by distilled water. After 30 min absorbance was measured at 420 nm.

Quercetein equivalence per gram of flavonoids were calculated using standard calibration curve obtained by performing the above experiment using 1 mg/ ml of quercetein in distilled water. Analyte concentration of unknown samples is calculated and results were graphically represented in Figures 3, 4.

2.3.3 Radical Scavenging activity by using DPPH method

Various concentrations of extracts were added with 3 mlof DPPH solution in methanol. They should be kept in dark region for the reaction to complete. After the incubation period, mixtures were measured for absorbance at 517 nm using UV-Visible spectrophoto-meter against blank which were adopted by Koleva *et al.*,(2002). Results are represented graphically in Figure 5.

Percentage scavenging of all were calculated

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

Where 1 ml of solvent mixed with 3 ml of DPPH was taken as control. For interpretation of the data from DPPH method inhibition concentration (IC_{50}) parameter was used which was calculated by using the formula

$$IC_{50} \text{ value} = \frac{50 - C}{m}$$

Where,

C = Intercept

m = Slope

2.3.4 Reducing power Assay

Method followed for present work was of Jayanthi and Lalitha (2011).

10mg of extract was dissolved in 10 ml of respective solvent from which it is extracted to get 1mg/ ml concentration, which was further used to obtain varied concentrations.1 ml of plant extract and standard of different concentrationswere taken in clean and dry test tubes. Phosphate buffer and potassium ferricyanide solutions were added. After incubation at 50°C trichloro-acetic acid was added. After centrifugation supernatant were taken and diluted. Absorbance wasmeasured after adding ferric chloride solution 700 nm. Graph was plotted by taking concentration and absorbance as X and Y axis, respectively, for ascorbic acid. Results are represented in Figure 6,7.

3. Results

3.1 Phytochemical analysis of leaf, stem and callus extracts

Preliminary phytochemical analysis carried out for various extracts of stem, leaf and callus in different solvents are tabulated in Table 1.

Hexane extracts were tested for the presence of various phytoconstituents such as carbohydrates only in leaf extract; sterols, triterpenes and glycosides in stem extract and triterpenes in callus extracts. Chloroform extractscontains carbohydrates, sterols, triterpenes proteins and resins, leaf extracts contains alkaloids, carbohydrates, resins and glycosides followed by callus extract with carbohydrates, and triterpenes.Ethyl acetate extracts ofleaf contains sterols, tannins, carbohydrates and glycosides while sterols, triterpenes, tannins, phenols and flavonoids were present in stem extract, whereas callus extract of ethyl acetate consists of sterols, tannins phenols and flavonoids. Majority of the phytoconstituents are present in the methanol extract, aqueous extract and ethyl acetate extract of leaf, stem and callus extracts hence they have been tested further for antioxidant activities. Aqueous stem extracts consists of phytoconstituents such as tannins, flavonoids and phenolic compounds, whereasaqueous leaf extractsconsist ofsterols, tannins, flavonoids tannins, flavonoids, resins and phenolic compounds but aqueous extract of callus confirmed with presence of tannins, flavonoids, triterpenesand phenolic compounds. Methanol extract of leaf on the other hand showed positive results for phytochemicals such as sterols, tannins, saponins, flavonoids, carbohydrates,

glycosides and phenolic compounds, whereas methanolextract of stem gave positive results for majority of the phytochemicals tested such as sterols, triterpenes, saponins, alkaloids, tannins, flavonoids, carbohydrates proteins and phenolic compounds; callus extract of methanol consists of saponins, tannins, flavonoids, proteins and phenolic compounds. Since majority of the phytoconstituents are present in ethyl acetate, methanol and aqueous extracts of Stem, leaf and callus, those are subjected to further antioxidant activities.

Table 1: Phytochemical analysis of stem, leaf and callus extracts in various solvents (L-leaf extracts, S-young stem extracts and C-callus extracts).

Type of Extract	Hexane Extract			Chloroform Extract			Ethyl Acetate Extract			Methanol Extract			Aqueous Extract		
L – Leaf Extract S - Stem Extract	L	S	C	L	S	C	L	S	C	L	S	C	L	S	C
Sterols															
1. Salkowskii's Test	–	+	–	–	+	–	+	+	+	+	+	–	+	–	–
2. Libermann-Burchard's Test	–	+	–	–	+	–	+	+	+	+	+	–	+	–	–
Triterpens															
1. Salkowskii's Test	–	+	+	–	+	+	–	+	–	–	+	–	–	–	+
2. Libermann-Burchard's Test	–	+	+	–	+	+	–	+	–	–	+	–	–	–	+
Saponins															
1. Foam Test	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
Alkaloids															
1. Mayer's Test	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
2. Dragendroff's Test	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
3. Wagner's Test	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
4. Hager's Test	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–
Tannins															
1. Ferric chloride test	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+
2. Gelatin test	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+
Flavanoids															
1. Shinoda test	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
2. Ferric chloride test	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
3. Lead acetate test	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
Carbohydrates															
1. Molisch test	+	–	–	+	+	+	+	+	–	–	+	–	–	–	–
2. Fehling's test	+	–	–	+	+	–	+	–	–	–	+	–	–	–	–
3. Benedict's test	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–
Resins															
1. Terbidity test	–	–	–	+	+	–	–	–	–	–	–	–	+	–	–
2. Acetic anhydride test	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–
Proteins															
1. Biuret test	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–
2. Ninhydrin test	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
Glycosides															
1. keller-killiani test	–	+	–	+	–	–	+	+	–	+	–	–	–	–	–
Phenolic compounds															
1. Ferric chloride test	–	–	–	–	–	–	–	+	+	+	+	+	–	+	+
2. Lead acetate test	–	–	–	–	–	–	–	+	+	–	+	+	+	+	–

3.2 Total phenolic content (TPC)

Total phenolics determined in different extracts of different plant extracts of stem, leaf and callus in comparison with gallic acid revealed that phenolics are present in higher concentration in methanol extract of leaf with 35.02 GAE/g followed by methanolic extract of stem with 32.05 GAE/g and ethyl acetate extract of leaf with 25.05 GAE/g. Which are followed by ethyl acetate extract of leaf, aqueous extract of leaf and methanol extract of callus with the values 30.12 GAE/g, 27.76 GAE/g and 19.03 GAE/g,respectively, ethyl acetate extract of stem, aqueous extract of stem and aqueous callus extract of leafwas recorded to have least concentration of phenolics compared to the rest with the values 23.35 GAE/g, 19.53 GAE/g and 15.00 GAE/g, respectively, the same can be referred in Figure 1, 2.

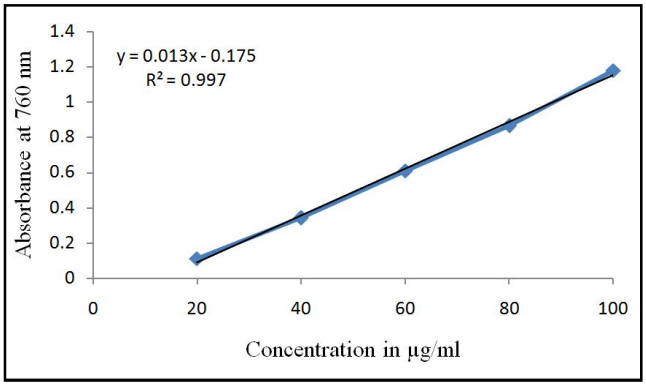

Figure 1: Calibration curve of Gallic acid.

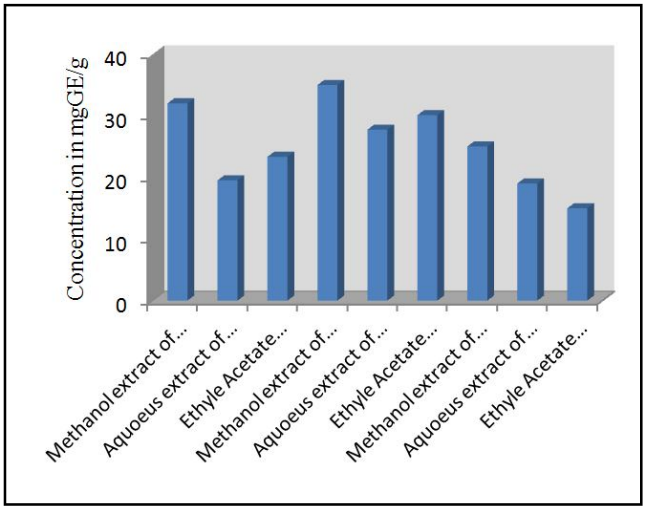

Figure 2: Comparison of total phenolic contents in various extract.

3.3 Total flavonoid content (TFC)

Figures 3, 4 depict the results of total flavonoid content assay with the standard flavonoid quercetein.Aqueous extract of stem showed the maximum flavonoid content with 628.57 mg/g of the plant extract, followed by ethyl acetate extract of leaf with 585.71 mg/g of the plant extract, then methanol extract of stem with 428.57 mg/g and 342.62 mg/g of aqueous extract of callus extract of the plant extract, the same can be seen in the Figure 2. Flavonoid content is found high in aqueous extract of stem may be because water is a polar solvent and has high affinity towards flavonoid contents. The same process is carried out for all other extracts as well but no significant results were found.

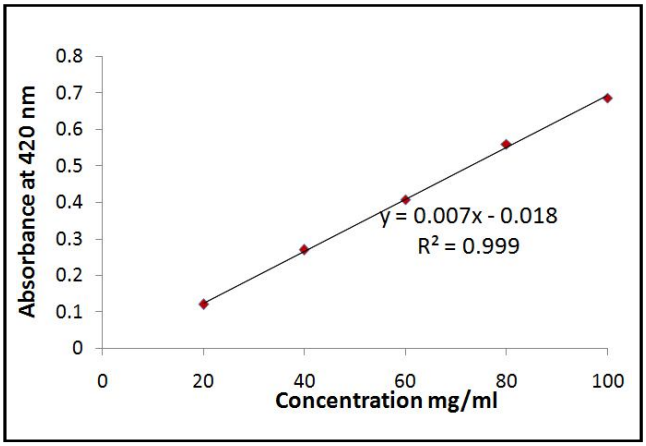

Figure 3: Calibration curve of Quercetein.

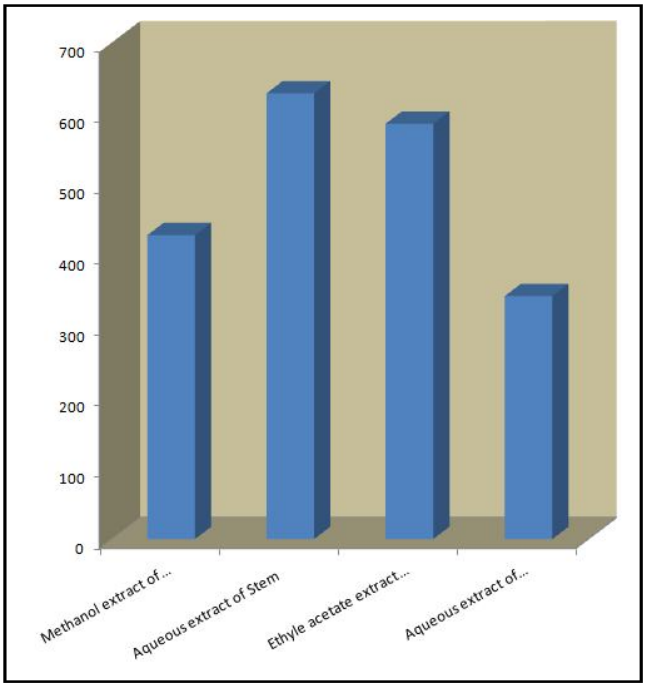

Figure 4: Comparison of total flavonoids in various extract.

3.4 Antioxidant activity of various extracts

There are many synthetic antioxidants which are in use and reported to have hazardous effects, whereas antioxidants are naturally present in plants and have beneficiary effects on health.DPPH assay is one of the well-known methods widely in use to examine the presence of antioxidants in different extracts; where model radicals are employed in evaluation of radical scavengers. Ethyl acetate extract, aqueous extracts and methanol extracts of all stem, leaf and callus extracts were tested for antioxidant activity.

3.5 DPPH assay of various extracts

Extracts exhibited increasing activity with increase in concentration from 20µg/ ml to 100µg/ ml and hence reported to have least activity

in 20µg/ ml and highest scavenging activity in 100µg/ ml. Leaf extracts of methanol have highest activity in 100µg/ ml with 96.16% compared to other extracts followed by methanol extracts of stem with 89.01% in 100µg/ ml concentration. Callus extracts showed least activities; among different extracts of callus, best scavenging activity was exhibited by methanol extract with 59.46% scavenging activity at 100µg/ ml concentration followed by aqueous extract and ethyl acetate extract with 47.93% and 38.28%, respectively, minimum scavenging percentage was 20% for callus in ethyl acetate extracts at 20 µg/ ml followed by 22.32%of activity in methanolic extracts of callus, whereas minimum methanolic scavenging activity for stem and leaf were quite high compared to callus with 28.46% for methanolic leaf extract and 35.14% for aqueous extracts of stem. From the present study it can be concluded that methanolic leaf extracts exhibited highest scavenging activity followed by stem and callus. The results can be seen in Figure 5.

IC_{50} value of ethyl acetate extract of stem is 34.29%, whereas leaf extract and callus extract in the same solvent gives36.79% and 55.36%, respectively,which can be compared with other values of different extracts, IC_{50} value of aqueous extract of stem is 85.51% which is comparatively very much greater than leaf and callus extract in same solvent i.e., 30.62 % and 70.89%, respectively,IC_{50} value of methanol extract of stem is 26.55% which is the least and infer having the best activity since the scavenging can occur with lesser quantity of sample i.e., it contains large quantity of antioxidants, IC_{50} value of leaf extract in methanol is 34.93% and as of callus is 59.02%. Figure 5 shows the results of percentage scavenging of methanol extract of stem and leaf.

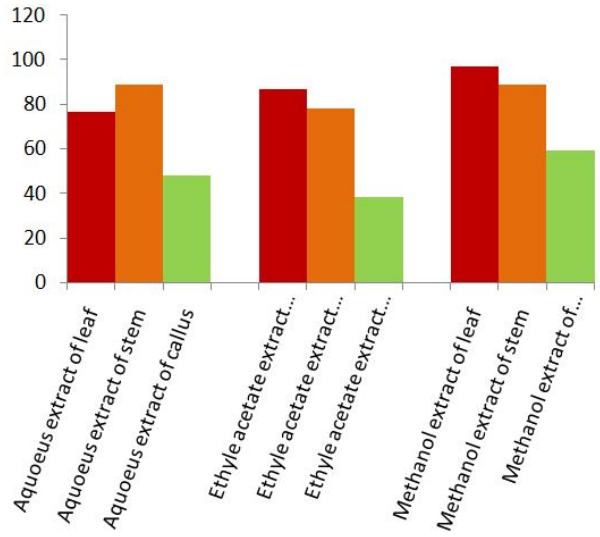

Figure 5: Comparison of percentage scavenging activity of different extract of leaf, stem and callus.

3.5 Reducing power assay of leaf, stem and callus extract in different solvents

Depending upon the reducing capacity of a sample, potential antioxidant activity can be determined. Antioxidants present in the sample are reductants which causes the reduction of ferricyanide complex to the ferrous form (*Wong et al*., 2006). This has been measured at 700 nm using spectrophotometer. Ascorbic acid was taken as standard for the comparison of reducing capacity of test samples.Reducing power assay of aqueous extract, methanol extract and ethyl acetate extract of stem, leaf and calluswere examined and methanolic extracts of both stem, leaf and callus extracts followed by aqueous exact were seemed to have more reducing power. This indicates that antioxidants are present abundantly in methanolic extract of both leaf and stem. Highest reducing power of 0.461 at 700 nm was found in stem extract of methanol in 100 µg/ mlconcentration, followed by 0.453at 700 nm for leaf in methanol extract and 0.253 for methanol extract of callus (Figures 6,7).

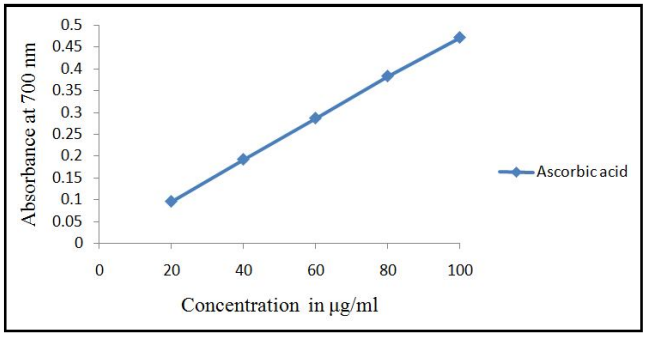

Figure 6: Reducing power assay of ascorbic acid (standard).

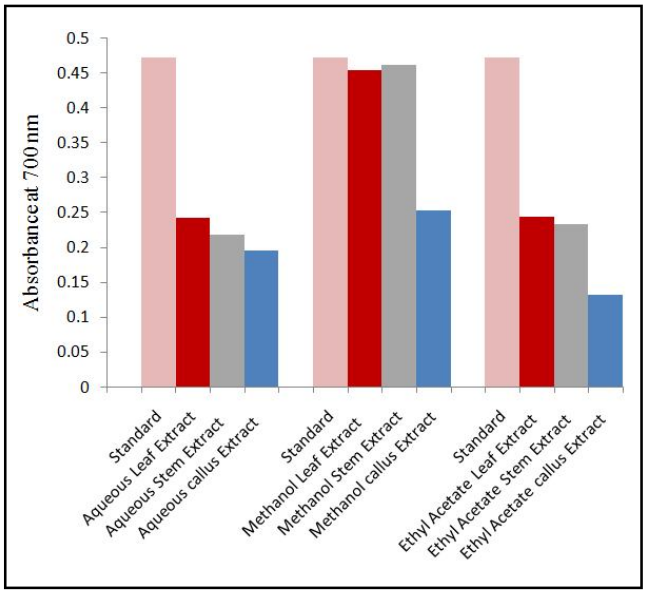

Figure 7: Comparison of reducing power assay of different extracts of leaf, stem and callus extracts.

4. Discussion

The results of Rani and Gopal's work on leaf and bark extracts of *P. indicum* in the year 2019 also reported with basic phytoconstituents as present work.Rani and Gopal's (2019) work on total phenolic content revealed that aqueous extracts of leaf consist of 36.37 GAE/ g and bark extracts of stem was reported to have high concentration of 47.70 GAE/g in ethanol extracts, whereas present study revealed highest concentration of phenols in methanolic extracts of leaf. Results of present study were contrast with Rani and Gopal's (2019) work which reported high flavonoids content in ethanol bark extract with 457.89 mg/g followed by aqueous bark extract with 252.63 mg/g.

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants are basically having scavenging activity and hence they are useful for the management of those diseases (Koleva *et al.*, 2002: Kumar *et al.*, 2008).Highest scavenging activity was in aqueous extract of leaf and ethanol extracts of bark.They also reported that both leaf and bark extracts of *P. indicum* have good reducing capacity in aqueous extracts. Similar studies carried out by Noungoue *et al*, (2006) on another species *Poeciloneuronpaciflourum*, which showed the presence of two new xanthones (1,6-dihydroxy-7-methoxyxanthone and 1,6-dihydroxy-7-methoxyxanthone 6-O-β-d-glucoside) responsible for antioxidant potential.

5. Conclusion

Among tested extracts of leaf, stem and callus of P. indicum, few were proved to have good antioxidant activity such as methanol extract of leaf and callus and aqueous extract of stem. Which were proved by screening followed by standardized antioxidant assays. Majority of phenolics were present in methanolic extracts. Total flavonoids were maximum in aqueous extract of stem, ethyl acetate extract of leaf and aqueous extract of callus.Scavenging activity also reflects the same. Results of present work conclude that plant consists antioxidant potentiality.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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