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PHYTOCHEMICAL SCREENING, IN VITRO ANTI-OXIDANT ACTIVITY AND IN VITRO ANTI-DIABETIC ACTIVITY OF LEAVES OF *PAEONIA EMODI* EXTRACT

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Abstract:

Free radicals contribute to more than one hundred disorders in humans. The synthetic antioxidants have been suspected to cause or prompt negative health effects leading to extensive research on naturally occurring antioxidants especially from plant sources. The aim of the present study was to evaluate qualitative and quantitative phytochemical constituents and in vitro antioxidant activities of leaves of Paeonia emodi. Antioxidant activity was carried out by using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) assay. The phytochemical screening of leaves of Paeonia emodi revealed the presence of Flavonoid, Phenol, Carbohydrates and Saponins in Hydroalcoholic extract. The percentage yield of Pet. ether and Hydroalcoholic extract of the leaves of Paeonia emodi was found to be 0.756, 2.524% w/w. The total phenolic and flavonoids content of Paeonia emodi leaves of hydroalcoholic extract was found to be 0.965 and 1.214mg/100mg respectively. The results of DPPH scavenging activity for leave ethanolic extract showed IC_{50} value 82.22, when compared to Ascorbic acid (standard) was 17.68. anti diabetic activity the IC50 values were examined 35.33µg/ml for Acarbose and 279.39µg/ml for Paeonia emodi extract. It indicates the plant has the potency of scavenging free radicals and antidiabetic potential it may provide leads in the ongoing search for natural antioxidants from various medicinal plants to be used in treating diseases related to free radical reactions.

Keywords: *Paeonia emodi, Phytochemical Screening, Antioxidant activity, Antidiabetic*

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INTRODUCTION:

Herbal medicines and their preparations have been widely used traditionally, for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drugs. One of the characteristics of oriental herbal medicine preparations is that all the herbal medicines, either presenting as single herbs or as collections of herbs in composite formulae (Balammal *et al.,* 2012). The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute mainly those traditional medicines which primarily use medicinal plant preparations for therapy. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials. (Kashaw *et al.,* 2011)

Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, osteoarthritis, diabetes, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Partap *et al.,* 2012).

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. Oxygen is absolutely essential for the life of aerobic organism but it may become toxic if supplied at higher concentrations. Dioxygen in its ground state is relatively unreactive; its partial reduction gives rise to active oxygen species (AOS) such as single oxygen, super oxide radical anion, hydrogen peroxide etc. This is partly due to the oxidative stress that is basically the adverse effect of oxidant on physiological function (Hall, 2001).

Free oxygen radicals plays cardinal role in the etiology of several diseases like arthritis, cancer, atherosclerosis etc. The oxidative damage to DNA may play vital role in aging and the presence of intracellular oxygen also can be responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause damage to critical cell bio-molecules (Satyavati *et al.,* 1976).

In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of plat materials act as radical scavengers, and convert the radicals to less reactive species (Devi and Ganasoundari, 1999). Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. The antioxidative system includes both enzymatic and non-enzymatic systems. The non enzymatic system includes ascorbic acid (vitamin C); ά-tocopherol, cartenes etc. and enzymic system include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and polyphenol oxidase (PPO) etc.

Paeonia emodi is a useful plant found in Western Himalayan region and is used in many ways by the locals and the scientists. It has various physiological activities including prevention of epileptic attacks, for cholera and whooping cough (watt, 1982). The tuber of the plant is highly effective medicine for uterine diseases, blood purifier, colic, bilious, backbone ache, headache, dizziness, vomiting, dropsy, epilepsy and hysteria while the seeds are Purgative. An infusion of the dried flowers is given to control diarrhea. In this study evaluation of different *in vitro* biological activities like antioxidant activity and antidiabetic activity of hydroalcoholic leaves extract of *Paeonia emodi*.

MATERIAL AND METHODS:

Material

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Methods

Collection of Plant material

Leaves of *Paeonia emodi* were collected from local market of Bhopal in the month of February, 2020.

Defatting of plant material

45.6 gram of leaves dried powdered of *Paeonia emodi* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

Defatted dried powdered of *Paeonia emodi* has been extracted with hydroalcoholic solvent (ethanol: water, 80:20) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007; Kokate, 1994).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical Screening

Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Roopashree *et al.,* 2008; Obasi *et al.,* 2010; Audu *et al.,* 2007).

Quantitative studies of phytoconstituents

Total phenol content estimation

Principle: The total phenol content of the extract was determined by the modified folin-ciocalteu method (Olufunmiso and Anthony, 2011).

Preparation of Standard: 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol.

Preparation of Extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol.

Procedure: 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for

colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principle: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Antioxidant activity of hydroalcoholic extract of leaves of *Paeonia emodi* using different model

DPPH method

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly (Olufunmiso and Anthony, 2011). Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Calculation of % Reduction = (Control Absorbance - Test absorbance) / (Control Absorbance) X100

In vitro* anti-diabetic activity of hydroalcoholic extract of leaves of *Paeonia emodi

Inhibition of alpha amylase enzyme

Preparation of standard: 10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 1000µg/ml were prepared in methanol.

Preparation of sample: 10 mg of dried extract was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition.

Method: A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

RESULTS AND DISCUSSION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Plants have great potential uses, especially as traditional medicine and pharmacological drugs. A large proportion of the world population depends on traditional medicine.

% Yield of leaves of hydroalcoholic extract of *Paeonia emodi* was found 2.524% as shown in Table 1. Phytochemical components such as phenolics, flavonoids, tannins, carbohydrates and saponins were determined in hydroalcoholic extract as shown in Table 2. Phytochemical analysis revealed that flavonoids, phenol, carbohydrates, saponins were found to be present in crude hydroalcoholic extract of *Paeonia emodi* and alkaloids, glycosides, proteins, diterpenes and tannins are absence. Table 3 shows the phenolic content with respect to gallic acid was found to be 0.965 (mg /100mg of extract) for of leaves of hydroalcoholic extract. The total flavonoids content of the extract was estimated taking quercetin as standard. The flavonoid content was found to be as: 1.214 (mg /100mg of extract) in hydroalcoholic extract.

Table 4 shows the results of antioxidant screening test for hydroalcoholic extract of *Paeonia emodi* using DPPH method. The comparative radical scavenging effect of standard and extract. The ascorbic acid and extracts have shown dose dependent scavenging of DPPH radicals. The radical

scavenging effect of standard and extracts was in the order ascorbic acid> leaves extract IC_{50} (µg/ml) was found to be 17.68 and 82.22 respectively. In anti diabetic activity the IC50 values were examined 35.33µg/ml for Acarbose and 279.39µg/ml for *Paeonia emodi* extract Table 5.

Table 1: % Yield of *Paeonia emodi*

S. No.	Extract	% Yield (w/w)
1.	Pet. ether	0.756
2.	Hydroalcoholic	2.524

Table 2: Phytochemical screening of extracts *of Paeonia emodi*

S. No.	Constituents	Hydroalcoholic extract	Observation
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve -ve	 Green coloured Not yellow coloured
2.	**Glycosides** Legal's test	 -ve	 Green coloured
3.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve	 Yellow colour precipitate Yellow colour
4.	**Phenol** Ferric chloride test	 +ve	 Black coloured
5.	**Proteins** Xanthoproteic test	 -ve	 Green coloured
6.	**Carbohydrates** Fehling's test	 +ve	 Red colour precipitate
7.	**Saponins** Foam test	 +ve	 Layer of foam
8.	**Diterpenes** Copper acetate test	 -ve	 Green coloured
9.	**Tannins** Gelatin Test	 -ve	 Green coloured

Table 3: Estimation of total phenolic and flavonoids content of *Paeonia emodi* extract

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	**Hydroalcoholic**	0.965	1.214

Table 4: % Inhibition of ascorbic acid and Hydroalcoholic extract of *Paeonia emodi* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	18.51
2	20	48.62	23.14
3	40	65.34	30.89
4	60	69.65	38.62
5	80	77.41	47.45
6	100	84.13	59.98
IC_{50} (µg/ml)		**17.68**	**82.22**

Table 5: % Inhibition of acarbose and Hydroalcoholic extract of *Paeonia emodi*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	*Paeonia emodi* extract
1	100	51.19	35.74
2	200	70.10	41.52
3	300	74.20	53.74
4	400	85.18	60.01
5	500	88.75	67.51
IC_{50} (µg/ml)		**35.33**	**279.39**

CONCLUSION:

The Hydroalcoholic leaves extract of *Paeonia emodi* had the best antioxidant effects against the DPPH. This suggests that *Paeonia emodi* can be used to prevent and control the oxidative stress induced by free radicals. The antidiabetic activity was also investigated, focusing on the inhibitory effects on α-Glucosidase. Our study is the first to report a potential mode of action of *Paeonia emodi* and suggests that the effect of this plant is due to the inhibition of digestive enzymes. On the other hand, the presence of flavonoids and phenols concludes that this herb has multiple biological properties. Other studies must be conducted to isolate the active ingredients of this plant, identify them, and study their bioactivity.

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