

A COMPARATIVE ANALYSIS OF PHYTOCHEMICAL AND ANTIOXIDANT PROFILE OF PAEDARIA FOETIDA.L WILD AND CULTIVATED VARIETIES

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ABSTRACT

Paederia foetida L. is one of the vulnerable medicinal plants of Odisha, which is collected from the wild. In the present study a comparison was made between the wildly collected variety with that of cultivated variety for its phytochemical and antioxidant potential. Phytochemical profiling of both revealed the presence of saponins, tannins and phlobotanins in the polar extracts. Anti oxidant activity was ascertained using DPPH and FRAP assays. Significant antioxidant activity to the tune of 84-85% was obtained in both the sample in DPPH assay. Comparative analysis of phenolic contents of wild and cultivated variety had no significant variations i.e., cultivated-3.044mg GAE/ml and wild-3.016mg GAE/ml. Concentration of flavonoids in wild was 0.647mg QE/ml which was comparable with the cultivated

variety i.e., 0.627mg QE/ml. Thus, all the above parameter showed wild and cultivated samples at par with each other in most of the medicinal properties.

KEYWORDS: Saponins, tannins, phlobotanins, DPPH assay, FRAP assay.

INTRODUCTION

Paederia foetida L. is commonly known as Ghandhali across India and as Pasaaruni in Odisha, is one of the species enlisted in the vulnerable category of medicinal plant for Odisha. In folklore system of medicine it is reported to be used in the treatment of gout, diarrhea, piles, dysentery, calculi, stomachic, emetic, ulcers and different type of inflammations.^[1] Besides that, It has also been reported for antiviral, antidiarrhoeal, anti-inflammatory^[2] and anti cancer activity.^[3] Owing to its multiple uses, same is collected from

the wild and which is the reason for its being vulnerable. In order to bring it in the main stream of cultivation, this study was taken up to explore the medicinal potential of cultivated variety of the plant and a comparison was drawn with that of the wildly collected samples using phytochemical and antioxidant parameters.

MATERIALS AND METHODS

2.1 Source of Plant material

The fresh and fully mature leaves of *Paederia foetida* L. were collected from both wild and cultivated areas. Cultivated source was the medicinal plant garden of RPRC, Nayapalli and wild variety was collected from Athantar square of Khurda district.

2.2 Preparation of plant extracts

The fresh leaves of *P. foetida* were washed with tap water immediately after collection and air dried for 4 - 5 days at room temperature. The collected leaves were chopped into small pieces and ground into coarse powder with a mechanical grinder (Usha, Lexus, India) and stored in an airtight container. Dried 20 g powder was taken in a thimble and run in soxhlet apparatus with the solvent hexane, chloroform, acetone and methanol respectively. All the extracted samples were dried under vacuum using rotary evaporator. Same process was repeated for both the samples.

2.3 Phytochemical analysis

Phytochemical tests were conducted using the standard protocols^[4]

2.4 Determination of total phenolic content

The total phenolic contents of the plant *Paederia foetida* L. of both the varieties were determined with some modification as described by standard protocols.^[5] Extracted samples of both the varieties (100µl) was mixed with 750µl of Folin Ciocalteu reagent (10 fold dilution with distilled water) and incubated at 25°C for 5 min. Then 750µl of sodium carbonate (60 g/L) solution was added to the mixture. Following 90 min incubation at 25°C, absorbance was measured at 725 nm using UV-visible spectrophotometer. The total phenolic content was measured using a standard curve of gallic acid at 0.02 – 0.1 mg/mL concentrations. Total phenolic content was calculated for each sample and expressed as milligrams of gallic acid equivalent per 100 mL of sample.

2.5 Determination of flavonoid content

The total flavonoid contents of the plant *Paederia foetida* L. of both the varieties were determined with some modification as per standard protocols^[5] One ml of 2% aluminium chloride was mixed with the same volume of sample at different doses and absorbance was measured at 430 nm after 10 min of incubation. The total flavonoid content was determined using a standard curve of quercetin at 20 – 100 µg/mL. Total flavonoid content was calculated for each sample and expressed as milligrams of quercetin equivalent per 100 mL of sample.

2.6 Antioxidant activity

2.6.1 Qualitative TLC based antioxidant activity

Thin layered chromatographic based DPPH assay^[6] was performed for each of the extracts of cultivated and wild varieties of *Paederia foetida* L. on Silica gel 60 coated plates, used as stationary phase.

Three mobile phases were used for each of the extracts.

- i) Benzene: Ethanol : Ammonium hydroxide (BEA)
- ii) Chloroform: Ethyl acetate: Formic acid (CEF) and
- iii) Ethyl acetate: Methanol: Water (EMW).

Used plates were first air dried and then the chromatograms were sprayed with 0.2% 2, 2, diphenyl-1-picryl-hydrazyl in methanol as an indicator. The presences of antioxidant compounds were detected by yellow bands against a purple background on the TLC plates and R_f values were calculated.

2.6.2 Quantitative Free radical scavenging activity

2.6.2.1 DPPH radical scavenging activity

DPPH radical scavenging activity was investigated according to the standard method.^[7] The scavenging effect was calculated using the following equation:

$$\text{Percentage of Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Where A_{control} is the absorbance of control

A_{test} is the absorbance of sample extracts.

IC₅₀ value was determined from the plotted graph of scavenging activity against the concentrations of the samples, which is defined as total antioxidant necessary to decrease the initial DPPH radical by 50%.

2.6.2.2 Ferric reducing power (FRAP) assay

FRAP reagent was prepared as described by Benzie and Strain.^[8] Different concentrations of extract and standard (62.5, 125, 250, 500, 1000 µg/ml) were prepared and solutions were obtained by adding 0.5ml from each concentrations with 1.5ml of FRAP reagent. Then absorbance was taken immediately (t_0) at 593nm. Ascorbic acid was used as standard and the FRAP value was determined by plotting in a standard curve.

RESULTS AND DISCUSSION

Phytochemical analysis

The leaf extracts of *Paederia foetida* L. of both the varieties cultivated and wild were dark in colour with characteristic smell and oily consistency. Preliminary phytochemical screening showed the presence of same class of phytochemicals, these were namely flavonoids, saponins, tannins and phlobatannins (Table 1). This study is in confirmation with earlier studies.^[9]

Table 1. Comparative phytochemical analysis of *P. foetida* L. of both the varieties:

Phytochemical compounds	Fresh leaves		Hexane extract		Chloroform extract		Acetone extract		Methanol extract	
	C	W	C	W	C	W	C	W	C	W
Alkaloid	-	-	-	-	-	-	-	-	-	-
Flavonoid	-	-	-	-	+	+	+	+	+	+
Anthraquinone	-	-	-	-	-	-	-	-	-	-
Saponin	-	-	-	-	-	-	-	-	+	+
Terpenoids	-	-	-	-	-	-	-	-	-	-
Cardiac glycoside	-	-	-	-	-	-	-	-	-	-
Tannin	+	+	-	-	-	-	+	+	+	+
Phlobatannin	+	+	-	-	-	-	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-

C- Cultivated, W-Wild, (-) - Absent, (+) – Present

Total phenolic and flavonoid contents

Phenolics and flavonoids are two naturally occurring phenolic compounds having antioxidant activity they also possess other activities like anti apoptosis, anti aging, anti carcinogenic, anti-inflammatory activity due to the reducing capacity of phenols.^[10] Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides. They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition etc.^[11] Flavonoids are particularly

beneficial, acting as anti oxidants and giving protection against cardiovascular disease, their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals.^[12]

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.816x + 0.040$, $R^2 = 0.838$). The values obtained for the concentration of total phenols were expressed as mg of GAE/mL of extract. The values of total phenolic contents ranged from 0.063 to 3.044 mg GAE/mL (Fig.1). More concentration of phenolics was measured in methanolic and acetone extracts where as hexane and chloroform extracts had less concentrations of phenols. Though acetone extract showed highest concentrations of phenolic content but comparative analysis of their wild and cultivated variety had negligible variation i.e., cultivated-3.044mg GAE/mL and wild-3.016mg GAE/mL. Methanolic extracts showed a little variation in amount of phenolic content with 1.158mg GAE/mL in cultivated and 1.423mg GAE/mL in wild varieties.

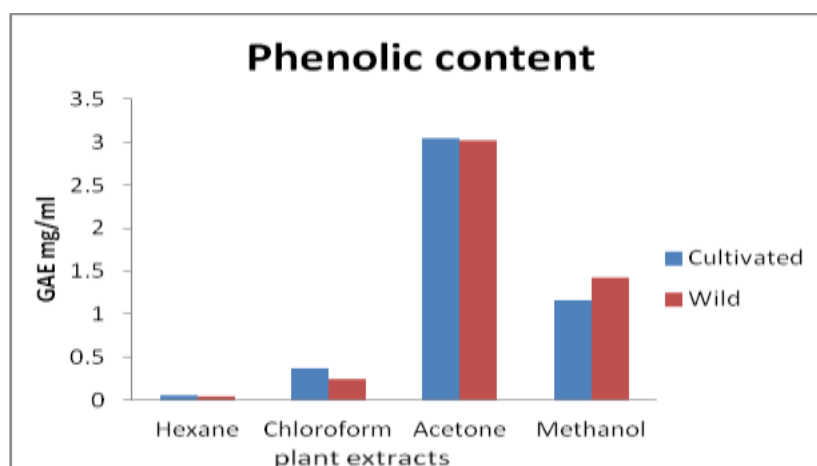


Figure 1: Total phenolic content of wild and cultivated solvent extracts

The concentration of flavonoids in various plant extracts of the species *P.foetida* L. was determined and the content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: $y = 11.89x + 0.100$, $R^2 = 0.997$), mg of QE/mL of extract. Total flavonoid contents in the plant extracts ranged from 0.03 to 0.647mg QE/mL. The highest concentration of flavonoids was measured in acetone (0.647mg QE/mL) extracts of cultivated variety where as hexane (0.14mg QE/mL), chloroform (0.36mg QE/mL) and methanolic (0.18mg QE/mL) extracts contain considerably smaller concentrations of flavonoids. In comparison to wild and cultivated varieties, hexane extracts showed remarkable differences

i.e., cultivated-0.03mg QE/mL and wild-0.14mg QE/mL. All other extracts had no such significant differences presented in Fig 2.

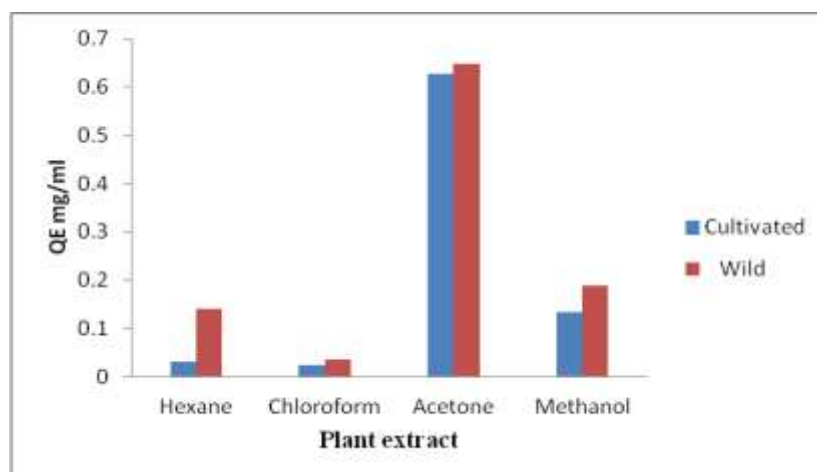


Figure 2: Total flavonoid content of solvent extracts

Antioxidant activity

Free radicals and reactive oxygen species are generated in living cells as a result of physiological and biochemical processes and these are causative agents for many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases in humans usually due to oxidative damage of proteins, lipids and DNA.^[13] Harmful effects of disturbed antioxidant can be largely prevented by intake of antioxidant substances.^[14] As can be seen in the Table 2, only in one of the extract i.e., methanol extracts of cultivated showed more number of antioxidant bands, where as in all the other extracts same number of antioxidant bands was obtained. Thus, profile of wild and cultivated in TLC based antioxidant assay was almost similar.

Table 2: TLC based antioxidant assay of *Paederia foetida* L. showing no. of antioxidant bands.

PLANT EXTRACTS	HEXANE		CHLOROFORM		ACETONE		METHANOL	
	C	W	C	W	C	W	C	W
BEA	5	5	7	7	5	5	1	1
CEF	2	2	2	2	STREAK	STREAK	5	4
EMW	NO	NO	NO	NO	6	6	4	2

The DPPH radical is considered as the a model of lipophilic radical, a chain reaction in lipophilic radicals was initiated by lipid auto oxidation^[15] and the radical scavenging activity of plant extract is determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH free radical. Among all the extracts acetone extract proved as a better free

radical scavenger comparable to standard ascorbic acid (Fig 3). But in comparison level both the varieties provided around the same percentage of inhibition i.e., C-84.84% and W-85.18%. IC₅₀ value of cultivated variety (116.6 µg/ml) was also close to the wild (121.21 µg/ml) one. Remaining extracts had mild activity. Ferric oxide reducing power assay was the only antioxidant assay which showed that all the extracts of cultivated variety were superior to the wildy collected samples (Fig 4). Thus, it can be concluded that cultivated samples of the *Paedaria foetida* can be successfully brought under main stream of cultivation as they are at par with their counterparts.

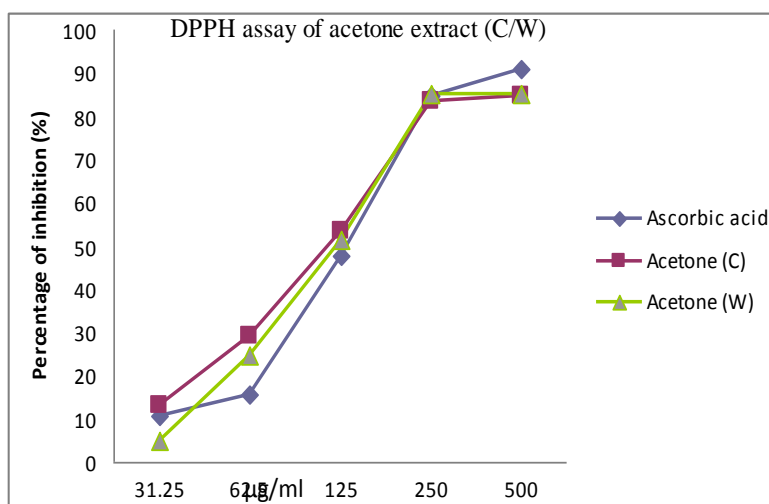


Figure3: DPPH radical scavenging assay

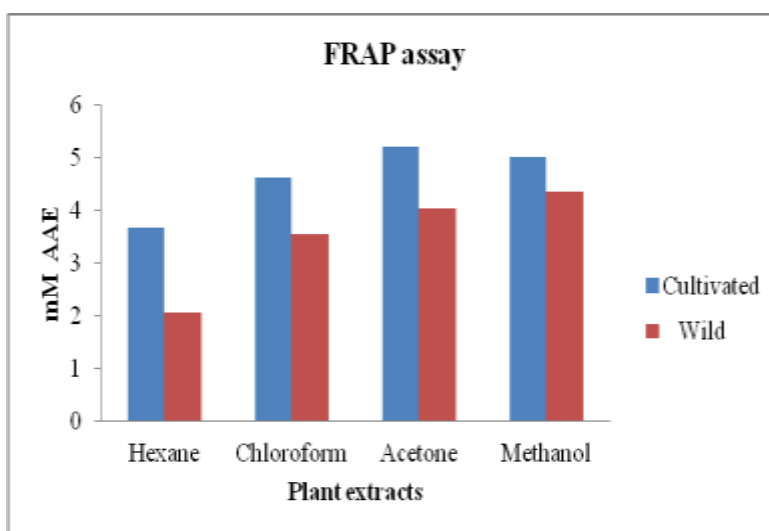


Figure4: FRAP assay of *P.foetida* L. (Cultivated and Wild)

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