



Comparison of *in vitro* antioxidant potential of fractioned *Paederia foetida* leaf extract

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Abstract:

Objective: The aim of this study was to investigate the antioxidant activity of different fractionated extracts of leaves of *Paederia foetida* by DPPH* scavenging assay and phytochemical analysis of the methanolic extract of the leaf. **Materials and Methods:** The fresh leaves of *P. foetida* was extracted by using methanol and then proceed for solvent fractionation. The extract was tested for phytochemical analysis and antioxidant activity using DPPH* scavenging assay. **Results:** Methanolic extract showed the presence of triterpenoids, volatile oil, sterols, alkaloid and glycoside. The yield of methanolic extract was 36%w/w which showed the highest antioxidant activity as compared to other fractions. **Conclusion:** *Paederia foetida* leaf extracts possessed free radical scavenging activity

Keywords: *Paederia foetida*, DPPH, Antioxidant

Introduction

Reactive oxygen species (ROS), e.g., superoxide radicals, hydroxyl radicals, and hydrogen peroxide, act as a significant causative factor for aging, (1) cancer (2,3) cardiovascular disease (4), inflammation, atherosclerosis, stroke, diabetes, Alzheimer's disease (5) etc. Studies reveal there is a correlation between high intake/high blood levels of antioxidants and low incidence of different types of cancer (6). Antioxidant compounds that scavenge free radicals help to protect against different kind of degenerative diseases (7). Plants, as the source of medicine, have been playing an important role to promote our health. Recently, there are several research

has been conducted to search potent natural antioxidant as it provides a protective effect against biological damages due to free radicals. The search for potent natural antioxidants, which act as a food as well as medicine, has become an important research issue at a world-wide level. One such plant which has the dual effect, i.e. as food and as medicine is *Paederia foetida* belonging to the family rubiaceae. It is an extensive foetid climber found in the Himalayas from Dehra Dun eastwards up to an altitude of 1800 m and also in Bihar, Orisa, Bengal and Assam (8). It has been advocated for various uses in the Indian systems of medicine as well as in folk-lore medicine. It possesses various compounds that are responsible for its diverse activities. Iridoid

glucosides namely asperuloside, scandoside and paederoside are the main chemical constituents present in the aerial part of the plant (9). It has a diverse pharmacological and phytochemical importance and extensive use by the tribal people of northeast as a vegetable and as well as medicine. Certain ethnic communities of Orissa, India cooks the leaves with rice to cure rheumatism and gout (10). Tribal of Tripura uses the leaves with dry fish (11). Traditionally, the prime use of the plant in different digestive problems like gastric trouble, to clean stomach, against stomach swelling, gastritis, in loose motion, diarrhoea, in indigestion, ulceration etc. (12-23). Afroz *et al.* (24) reported its anti diarrhoeal property. There are several other studies which established its hepatoprotectivity (25-28). The other therapeutic uses are antitussive (29), anti-arthritic (30), anti-inflammatory (31), analgesic (32) etc. Thus, the objective of this research was to investigate the antioxidant activity of leaf (fresh) extract and it's fractioned.

Materials and Methods

Collection and authentication of plant material

Leaf of *Paederia foetida* Linn. (Rubiaceae) was collected from the local tribal market of Agartala, Tripura. The prepared herbarium was submitted to the National Institute of Science Communication, New Delhi (authentication Ref No. NISCAIR/RHMD/Consult/2010-11/1442/40) for authentication.

Extraction and fractionation of leaf of *P. foetida*

After the pharmacognostic identification, the fresh material (500gm) was extracted with methanol (1.5l) in a Soxhlet apparatus. The methanolic extract (PFM) was collected by filtration, evaporated till dryness on a rotary

evaporator. PFM was proceeding to solvent fractionation in two ways. Firstly, the dried PFM was suspended in water, followed by solvent fractionation by pet ether, toluene and chloroform. The remaining aqueous layer was freeze dried and the residue (RAL) so obtained was kept in a vial for further use. On the other way the residue of PFM was dissolved in 5% acetic acid (pH1) and fractioned with dichloromethane. The aqueous acidic layer was basified with 10% sodium carbonate (pH 10) and further fractioned with dichloromethane. So obtained an aqueous layer was concentrated on a water bath to its 50% of the volume followed by freeze dried (ALK-Aqa-1) and kept in refrigerator for further use.. All the sample, i.e. PFM, RAL and ALK-Aqa-1 were subjected to the evaluation of antioxidant activity.

Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. Rutin (Ozone, Mumbai) was used as a standard drug. All other chemicals/solvent used were of analytical grade.

Phytochemical screening

The Phytochemical screening of methanol extract was done to identify the main groups of chemical constituents present in the methanol extract of *P. foetida* by their color reaction.(33)

DPPH* radical scavenging activity

The free radical-scavenging activity of different extract of *P. foetida* was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. The lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. DPPH assay was carried out as per the method of (34). In brief, a 250µl total reaction volume contains 10µl of DPPH solution; various concentrations of

test solution and sufficient volume of methanol to make 250 μ l. The reaction mixture was mixed and incubated at 25°C for 20 min following which the absorbance was read at 510nm using microwell plate reader. A control reaction was carried out without the test sample.

Calculation:

$$\% \text{ inhibition} = \frac{\text{Abs of control} - \text{Abs of test}}{\text{Abs of control}} \times 100$$

RESULTS

Phytochemical Screening: The yield of alcohol free methanol extract was 36%w/w on dry basis.

Table 2: DPPH* radical scavenging activity of PFM, RAL and ALK-Aqa-1

Concentration (μ g/ml)	Sample: PFM % of inhibition (Mean \pm SEM)	Sample: RAL % of inhibition (Mean \pm SEM)	Sample: ALK-Aqa-1 % of inhibition (Mean \pm SEM)
10	21.94 \pm 1.631	16.15 \pm 1.369	8.12 \pm 0.821
20	37.84 \pm 0.9601	25.8 \pm 0.855	15.68 \pm 1.18
40	50.57 \pm 1.133	38.17 \pm 0.664	26.58 \pm 0.522
80	69.18 \pm 2.061	57.08 \pm 0.871	37.58 \pm 1.369
100	80.06 \pm 1.031	65.67 \pm 0.71	43.13 \pm 1.654
120	80.79 \pm 1.110	66.67 \pm 0.202	43.35 \pm 1.790
Blank	-	-	-

Conclusion

From the current study, we can conclude that *P. foetida* extract has high antioxidant activity and free radical scavenging activity could be attributed to the presence of flavonoids, tannins, glycosides and phenolic compounds. We can also conclude that the antioxidant activity is higher in methanolic mother extract rather than in the other two fractions (RAL and ALK-Aqa-1). This assay proof the important applications of the plant for the food and as well as in the pharmaceutical industry and hereby concrete the ethnomedicinal uses.

Reference

Phytochemicals of the methanol extract of the plant are listed in Table no 1.

Table 1: phytochemicals of methanolic extract

Test compound	Result
Glycoside	Present
Alkaloid	present
Sterols	Present
Volatile oil	Presents
Tannins	Absent
Triterpenoids	Present

The anti oxidant activity of methanolic extract is higher (table 2) when compared to the other fractioned sample (RAL and ALK-Aqa-1) of *P. foetida* which showed the less percentage of inhibition (table 2)

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