



# 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 $\mu$ 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 ( $\mu$ 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)

- 1) Finkel T, Holbrook NJ. (2000) Oxidants. Oxidative stress and the biology of aging. Nature 408:239–247.
- 2) Pietta PG. (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042.
- 3) Cerutti PA. (1994) Oxy-radicals and cancer. Lancet 344:862–863.
- 4) Frei B. (1995) Cardiovascular disease and nutrient antioxidants: role of low-density lipoprotein oxidation. Crit Rev Food Sci Nutr 35:83–9.
- 5) Devasagayam TPA, Tilak JC, Bloor KK et al. Review: Free radical and antioxidants in human health. Curr Stat Fut Pros JAPI 53: 794-804, 2004.
- 6) Suzuki T, Wakai K, Matsuo K, Hirose K, Ito H, Kuriki K, Sato S, Ueda R, Hasegawa Y, Tajima K. (2006) Effect of dietary antioxidants and risk of oral,pharyngeal and laryngeal squamous cell

- carcinoma according to smoking and drinking habits. *Cancer Sci.* 97:760–767.
- 7) Amin I, Tan SH. (2002) Antioxidant activity of selected commercial seaweeds. *Malays. J. Nutr.* 8:167-177.
  - 8) Nadkarni KM. 1982. *The Indian Materia Medica*. 3<sup>rd</sup> ed. Popular Prakashan Pvt Ltd; Bombay.
  - 9) Shukla YN, Lloyd HA, Morton JF, Kapadia G. (1976) Iridoid glycosides and other constituents of *Paederia foetida*. *J. Phytochemistry* 15:1989-1990.
  - 10) Singh H, Krishna G, Baske PK. (2010) Plants used in the treatment of joint diseases (rheumatism, arthritis, gout and lumbago) in Mayurbhanj district of Odisha, India. *Report and Opinion* 2:22-26.
  - 11) Das P. 1997. *Wild tribal plants of Tripura tribes*. Tripura Tribal Cultural Research Institute & Museum; Govt of Tripura, Agartala.
  - 12) Barua U, Hore DK, Sarma, R. (2007) Wild edible plants of Majuli island and Darrang district of Assam. *Indian Journal of Traditional Knowledge* 6:191-194.
  - 13) Chanda, S., De, B., Tiwari, R.K., 2011. Traditional and ethnobotanical investigation of some edible plants among the tribes of Tripura, India, in: Choudhury, M.D., Sharma, G.D., Choudhury, S., Talukdar, A.D., Status and conservation of bio-diversity in North East India. Swastik publications, Delhi pp.118-124.
  - 14) Kagyung R, Gajurel PR, Rethy P, Singh B. (2010) Ethnomedicinal plants used for gastrointestinal disease by *Adi* tribes of Dehang-Debang Biosphere Reserve in Arunachal Pradesh. *Indian Journal of Traditional Knowledge* 9:596-601.
  - 15) Kala CP. (2005) Ethnomedicinal botany of the Apatani in the Eastern Himalayan region of India. *Journal of Ethnobiology and Ethnomedicine* 1:11.
  - 16) Kar A, Borthakur SK. (2008) Wild vegetables of Karbi-Anglong district Assam. *Natural product Radiance* 7:448-460.
  - 17) Pfoze NL, Kumar Y, Myrboh B. (2012) Survey and assessment of ethnomedicinal plants used in Senapati District of Manipur State, Northeast India. *Phytopharmacology* 2:285-311.
  - 18) Reddy KN, Pattanaik C, Reddy CS, Raju VS. (2007) Traditional knowledge on wild food plants in Andhra Pradesh. *Indian Journal of Traditional Knowledge* 6:223-229.
  - 19) Sarmah TR, Adhikari D, Majumdar M, Arunachalan A. (2008) Traditional medicobotany of *Chakma* community residing in the Northwestern periphery of Namdapha National park in Arunachal Pradesh. *Indian Journal of Traditional Knowledge* 7:587-593.
  - 20) Srivastava RC, Singh RK, Apatani community, Mukherjee TK. (2010) Indigenous biodiversity of *Apatani* plateau: Learning on biocultural knowledge of *Apatani* tribe of Arunachal Pradesh for sustainable live hoods. *Indian Journal of Traditional Knowledge* 9:432-442.
  - 21) Sumpam T, Nima DN, Chocha A, Anggu Litin. (2011) An ethnobotanical survey of medicinal plants in the Eastern Himalayan zone of Arunachal Pradesh, India. *J of Ethnopharmacology* 134:18-25.
  - 22) Rethy P, Singh B, Kagyung R, Gajurel P. (2010) Ethnobotanical studies of Dehang-Debang Biosphere Reserve of Arunachal Pradesh with special reference to *Memba* tribes. *Indian Journal of Traditional Knowledge* 9:61-67.
  - 23) Chanda S, Sareth, IP, De B, Singh K. (2013) *Paederia foetida* - a promising ethno-medicinal tribal plant of northeastern India. *Journal of Forestry Research* 24:801-808.
  - 24) Afroz S, Almgir M, Khan MT, Jabbar S, Nahar N, Choudhuri MS. (2006)
  - 25) Antidiarrhoeal activity of the ethanol extract of *Paederia foetida* Linn. (Rubiaceae). *J Ethnopharmacol* 21:125-130.
  - 26) Uddin B, Nahar T, Basunia MA, Hossain S. (2011) *Paederia foetida* protects liver against hepatotoxin –induced oxidative damage. *Advances in Biological Research* 5:267–272.

- 27) De S, Ravishankar B, Bhavsar GC. (1993) Evaluation of *Paederia foetida* for Hepatoprotective and Anti-inflammatory Activities. *Indian Journal of Natural. Product* 9:7–10.
- 28) De S, Shukla VJ, Ravishankar B, Bhavsar GC. (1996) A preliminary study on the hepatoprotective activity of methanol extract of *Paederia foetidaleaf*. *Fitoterapia* 67:106–109.
- 29) Yang LL, Yen KY, Kiso Y, Kikino H. (1987) Antihepatotoxic actions of Formosan plant drugs. *Journal of Ethnopharmacology* 19:103–110.
- 30) Nosalova G, Mokry J, Ather A, Khan MTH. (2007) Antitussive Activity of the Ethanolic Extract of *Paederia foetida* (Rubiaceae family) in Non Anaesthetized Cats. *Acta Veterinaria Brno* 76:27–33.
- 31) Rajashekhara N, Sharma PP, Vasanth P. (2009) Comparative study of Prasaraani (*Merremia tridentata* Hallier. f. (Convolvulaceae) and *Paederia foetida* Linn. (Rubiaceae)) in Amavata (Rheumatoid Arthritis) *Ayu* 30:503-507.
- 32) Srivastava MC, Tewari JP, Kant V. (1973) Anti-inflammatory activity of an indigenous plant – *Paederia foetida* (Gandhali). *Indian Journal of Medical Sciences* 27:231–234.
- 33) Hossain M, Mohammad SA, Saha A, Alimuzzaman M. (2006) Antinociceptive activity of whole plant extracts of *Paederia foetida*. *Journal of Pharmaceutical Sciences* 5:67–69.
- 34) Khandelwal KR. 2008. *Practical Pharmacognosy: Techniques and Experiments* 19<sup>th</sup> Eden, Nirali Prakashan; Pune.
- 35) Vani T, Rajani M, Sarkar S, Shishoo CJ. (1997) Antioxidant properties of the Ayurvedic formulation Triphala and its constituents. *Inter. J. Pharmacognosy* 35:313

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