IDENTIFICATION OF ADULTERANT SPECIES, CHEMOTYPING AND *EX SITU* CONSERVATION OF *URARIA PICTA* FROM THE GANDHAMARDAN, ODISHA

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Abstract

The plant species *Uraria picta*, commonly known as Prsniparni, belongs to the family Fabaceae is in high demand by several Ayurvedic, pharmaceutical and herbal medicine industries. It is reported to be a perennial erect woody herb reaching approximately 1.0–2.5 m in height. *U. picta* is one of the essential constituents of 'Dasamula' (10 root plants) which is used for the treatment of fever and inflammation. Mostly the roots of *U. picta* are used in formulations other than of Dasamula of ayurveda such as amrtarishta, sirah, suladi, vajra, rasa, etc., and in many instances also used as single drug (dasamula taila, dasamularishta). All parts of this plant have medicinal importance and are used by certain Adivasis and native tribes. Leaves are good antiseptics and are used against gonorrhoea. Fruits and pods are effective against oral sores in children, and roots are used against cough, chills, and fever. The roots are used medicinally for invigorating the liver and spleen as sedatives to produce strength to the nervous system, cure all types of inflammation, detoxify the entire body, be used to treat skin diseases and heal fracture wounds. Traditionally, the plant is used as an antidote against the bites of certain Indian vipers. The root isolates of the plants show antimicrobial activity against fungi and both Gram-positive and Gram-negative bacteria. Pharmacologically, the plant is used for the treatment of UTIs (urinary tract infections), edema, tumors, etc., because of the presence of several phytochemical constituents, such as phenols, flavonoids, and terpenoids. It also exhibits anti-inflammatory activities, hepatoprotective effects, anti-acaricidal activity, antimicrobial efficacy, antinociceptive effects, antioxidant activity, fracture healing activity, anticancer activity, protective effects, antidiabetic activity, and anxiolytic activity in the treatment of Alzheimer's disease. *U. picta* is becoming increasingly rare because of overexploitation by various pharmaceutical industries as well as local tribes for medicine and trade purposes coupled with poor seed viability. Specifically, high consumption of raw material of *U. picta* has resulted in two problems: (a) a reduction in its population to the extent that it has been declared endangered and (b) adulteration of its plant material with less expensive plant species, which affects the quality and efficacy of herbal drugs.The trade value and economics of *Uraria picta* is largely affected by adulterants of other species such as *Dsemodium gangaticum, D. puchellum, D. velutinum, D. longipes* and *L. reticulata*. The endangered status and high medicinal value of *U. picta* require immediate interventions to

develop strategies not only for its conservation and utilization but also for identifying elite chemotypes for large-scale multiplication.

The utilization of roots by uprooting the entire plant and the problems associated with seed germination threaten this plant in its natural habitat and cause a shortage of root material for Ayurvedic preparation. The present study aimed to assess seed viability and the influence of pre-sowing treatments on seed germination and seedling development of *U. picta*. The 2,3,5-triphenyltetrazolium chloride (TTC) test was employed in this study to assess seed viability; this test specifically targeted both nondormant and dormant seeds. Seeds of *Uraria picta* were subjected to the TTC test, where intact seeds showed no staining due to impermeability through the seed coat, whereas embryos of seeds cut at the micropylar end turned dark red, indicating 100% viability. However, germination did not occur under moist conditions, suggesting the presence of dormancy due to a hard seed coat. To enhance germination, various physical (sandpaper, hot water, cold water) and chemical (KNO_3, HCl, H_2SO_4) treatments were applied, with 90% sulfuric acid yielding the highest germination rates. Comparative in vitro studies on seed germination revealed that seeds treated with 90% H_2SO_4 followed by surface sterilization and inoculation on ½ MS media supplemented with 2.0 mg/L GA3 achieved the highest germination rate (90%). For shoot multiplication, explants cultured on MS media supplemented with cytokinins presented significant results, with the highest number of shoots (21 shoots per explant) and longest shoot length (7.1 cm) observed on media supplemented with 2.0 mg/L meta-topolin (mT). Root induction was successfully achieved when in vitro shoots were inoculated on ½ MS media supplemented with 1.0 mg/L IBA, resulting in a maximum of 7 roots per shoot. The regenerated plantlets were acclimatized, with a survival rate exceeding 95%. This study highlights the effectiveness of chemical treatments and the role of cytokinins in shoot multiplication and root induction for successful propagation of *Uraria picta*.

The formulation of Dashmoolarista by Ayurvedic science is a boon to modern society, and *U. picta* is one of the major ingredients. Owing to the increase in the demand for Dashmoolarista, companies have started to find adulterant species for *U. picta.* Most plant species, namely, *Dsemodium gangaticum, D. puchellum D. velutinum, D. longipes* and *L. reticulata,* are adulterant species in Dashmoolarista. Therefore, we have carried out the analysis of phytoconstituents of *U. picta* and compared with its adulterant species. *Uraria picta* has the highest phenol content (11.10 mg/g) among the five adulterant species, along

with high flavonoid (8.43 mg/g) and alkaloid (6.14 mg/g) levels, while its tannin (5.582 mg/g) and saponin (5.23 mg/g) contents are moderate. *Desmodium gangeticum* follows closely with a phenol content of 10.06 mg/g, slightly lower flavonoid (7.92 mg/g) and alkaloid (6.01 mg/g) levels, but a higher saponin content (6.13 mg/g) and similar tannin levels (5.408 mg/g) to *Uraria picta*. *Desmodium velutinum* generally has a relatively low phytochemical content, with the lowest phenol (7.89 mg/g), flavonoid (5.32 mg/g), and tannin (4.160 mg/g) contents, although its alkaloid (4.91 mg/g) and saponin (6.11 mg/g) contents are moderate. *Desmodium longipes* presented a balanced profile with moderate phenol (9.37 mg/g), flavonoid (6.12 mg/g), tannin (5.314 mg/g), alkaloid (5.67 mg/g), and saponin (6.01 mg/g) contents. *Desmodium puchellum* has phenol (10.01 mg/g) and flavonoid (7.89 mg/g) contents similar to those of *Desmodium gangeticum,* with high tannin (5.512 mg/g) and moderate alkaloid (5.89 mg/g) and saponin (5.81 mg/g) contents. Finally, *Leptadenia reticulata* presented the lowest phenol (6.81 mg/g), tannin (3.12 mg/g), and alkaloid (3.78 mg/g) contents but comparable flavonoid (6.81 mg/g) and saponin (6.3 mg/g) contents to those of the other species. The DPPH assay revealed that *Uraria picta* had strong antioxidant capacity, with an IC_{50} of 10.17 µg/mL, which was close to that of the reference ascorbic acid (IC50: 9.59 µg/mL). Among the adulterants, *Desmodium gangetium* had moderate effects (IC_{50}: 12.73 µg/mL), whereas *Desmodium pulchellum*, *Desmodium longipes*, *Desmodium velutinum,* and *Leptedenia reticulata* had significantly weaker antioxidant activities, with IC_{50} values ranging from 30.78 to 46.94 µg/mL. In the FRAP assay, *Uraria picta* displayed exceptional antioxidant power (IC_{50}: 2.93 µg/mL), far exceeding that of ascorbic acid (IC_{50}: 250.07 µg/mL). *Desmodium gangetium* also presented moderate activity (IC_{50}: 5.72 µg/mL), whereas the other species presented much higher IC_{50} values, indicating weaker activity. The ABTS assay confirmed that *Uraria picta* has strong antioxidant activity (IC_{50}: 77.41 µg/mL) compared with ascorbic acid (IC_{50}: 122.44 µg/mL). *Desmodium gangetium* exhibited moderate activity (IC_{50}: 101.45 µg/mL), whereas the other species presented weaker antioxidant activity, with IC_{50} values ranging from 102.89 to 119.56 µg/mL. GC-MS analysis of the crude extract of *U. picta* and its adulterant species revealed a wide variation in their composition. LC–MS analysis revealed varying levels of Rhoifolin in *Uraria picta* and its adulterant species. *Uraria picta* presented the highest concentration at 0.31%, indicating the significant presence of this compound. In contrast, *Desmodium gangetium* had a very low Rhoifolin concentration of only 0.01%, and no Rhoifolin was detected *in Desmodium pulchellum, Desmodium longipes, Desmodium*

velutinum, or Leptedenia reticulate, indicating that these species either do not produce Rhoifolin or contain it in quantities too small for LC–MS detection.

Uraria picta and its adulterant species have several pharmacological activities, but despite their beneficial activities, they are considered adulterant species. This situation generally arises from their tendency to interfere, particularly in herbal medicine. As a result, the advantageous properties that *U. picta* can deliver may be unnoticed due to concerns about the quality and purity of the products they affect. Therefore, it is crucial to evaluate and manage these plant species carefully to ensure that their medicinal benefits can be utilized effectively while reducing their potential negative impacts. Here we have carried out a comparative evaluation of the antibacterial and antibiofilm efficacy against MDR bacteria, cytotoxicity to cancer cells and toxicity profile of *U. picta* and its adulterant species. Preliminary antibacterial screening of different extracts from *Uraria picta*, *Desmodium gangeticum, Desmodium pulchellum, Desmodium velutinum, Desmodium longipes*, and *Leptadenia reticulata* was conducted against ten clinically isolated bacterial strains, including *S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, E. coli, Shigella* sp., *P. mirabilis, S. typhi, S. paratyphi A,* and *S. paratyphi B. Uraria picta, Desmodium gangeticum,* and *Desmodium pulchellum,* which showed antibacterial activity against *S. aureus*, while *Uraria picta and Desmodium gangeticum* also exhibited significant activity against *E. coli*. The minimum inhibitory concentration (MIC) of *Uraria picta* and *Desmodium gangeticum* against *S. aureus* was 12.5 mg/mL, whereas for *Desmodium pulchellum,* it was 25 mg/mL. Both *Uraria picta* and *Desmodium gangeticum* presented MICs of 12.5 mg/mL against *E. coli*. The minimum bactericidal concentration (MBC) against *S. aureus* was 25 mg/mL for *Uraria picta*, 12.5 mg/mL for *Desmodium gangeticum*, and 50 mg/mL for *Desmodium pulchellum*. For *E. coli*, the MBC was 12.5 mg/mL for *Uraria picta* and 50 mg/mL for *Desmodium gangeticum*. At the MIC, *Uraria picta, Desmodium gangeticum,* and *Desmodium pulchellum* showed zones of inhibition of 15.4 ± 0.4 mm, 11.2 ± 0.9 mm, and 10.9 ± 0.7 mm, respectively, against *S. aureus*, whereas *Uraria picta* and *Desmodium gangeticum* showed inhibition zones of 13.2 ± 0.2 mm and 10.7 ± 0.3 mm, respectively, against *E. coli*. In comparison, the antibiotic imipenem had inhibition zones of 17.9 ± 0.7 mm and 16.2 mm against *S. aureus* and *E. coli*, respectively. At sub-MIC (1/2 MIC) concentrations, extracts from *Uraria picta, Desmodium gangeticum*, and *Desmodium pulchellum* significantly reduced exopolysaccharide production and biofilm formation in bacterial strains, as shown by tube adherence assays. The presence of

minor rings at the liquid–air interface and reduced attachment to surfaces indicated a decrease in biofilm matrix formation compared with that of the untreated control, which exhibited heavy rings and dense biofilms. This reduction was further confirmed through crystal violet staining and light microscopy, which revealed thinner, nonuniform biofilm matrices in the treated samples. In contrast, extracts from *Desmodium velutinum, Desmodium longipes, and Leptadenia reticulata* did not inhibit biofilm formation.

The cytotoxicity of *Uraria picta* and its adulterant species was assessed using the MTT assay on the MDA-MB-231, A549, and HEK-293 cell lines, revealing a dose-dependent effect across various concentrations (12.5 to 600 μg/mL). *Uraria picta* exhibited significantly greater cytotoxicity, particularly against cancerous cell lines, with approximately 50% viability at 167.1 μg/mL in MDA-MB-231 cells and 153.9 μg/mL in A549 cells. In contrast, the adulterant species required relatively high concentrations (400 μg/mL and above) to achieve similar effects. In noncancerous HEK-293 cells, both *Uraria picta* and the adulterant species demonstrated minimal cytotoxicity at lower concentrations, with *Uraria picta* showing moderate cytotoxicity at 600 μg/mL. These findings suggest that *Uraria picta* possesses superior anticancer activity while remaining relatively safe for normal cells, emphasizing its potential as an anticancer agent and the need for authenticity in medicinal formulations.

In a toxicity study, the effects of a crude extract of *Uraria picta* and its adulterant species on various health parameters were assessed in rats treated with a daily dose of 200 mg/kg body weight for 28 days. The results revealed no significant changes in body weight, water intake, or food intake compared with those of the untreated control group. Hematological parameters, including WBCs, HGB, RBCs, HCT, PCV, platelet count, MCV, MCH, and PCT, were not significantly different between the treated and control groups. Additionally, the serum levels of glucose, albumin, globulin, total protein, and electrolytes (sodium, potassium, calcium, and phosphorus) were consistent across both groups. Liver function parameters (ALT, AST, and ALP) and kidney function parameters (urea and creatinine) also remained unchanged. Histological examinations of vital organs, including the brain, heart, lungs, kidneys, spleen, and liver, revealed no significant alterations compared with those in the untreated group. Specifically, the liver was intact, the kidney structures were normal, the lung tissue displayed healthy alveoli, and the cardiac muscle fibres appeared unchanged, with the brain tissue remaining normal.

In conclusion, examination of the differences in phytochemical composition, quantification of the marker compound, rhoefilin and different pharmacological activities in terms of antibacterial and antibiofilm effectiveness against MDR bacteria, cytotoxicity against cancer cell lines and toxicity profiling of *Uraria picta* suggest a remarkable variation of *U. picta* in comparison to its adulterant species. Further, the study undertaken highlights the importance of *U. picta* to fight against multidrug-resistant bacteria, with the ability to interrupt biofilm formation, which is important for addressing persistent bacterial infections. This investigation highlights the importance of discovering natural products as feasible therapeutic agents, particularly in an era where conventional antibiotics are progressively losing their efficacy. Future studies should focus on isolating specific bioactive compounds, understanding their mechanisms of action, and leading clinical trials to assess their therapeutic potential. This work contributes to the increasing body of information designed to combat antibiotic resistance and endorses the sustainable use of herbal drugs in present healthcare.