Qualitative Phytochemical Screening And Metal Analysis Of Some Selected Medicinal Plants

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KEYWORDS

Medicinal plants, heavy metals, minerals, Atomic absorption spectroscopy, traditional medicine, phytochemical analysis.

Abbreviation: ppm - parts per million, AAS-Atomic Absorption Spectroscopy

ABSTRACT

Medicinal plants have been integral to traditional medicine system for centuries due to their therapeutic properties. By employing standard phytochemical screening methods and Atomic absorption spectroscopy, the selected medicinal plants like Entada rheedei, Tridax procumbens and Balanited aegyptiaca were screened for different phytochemicals and metals. By these analyses, we detected key constituents like flavonoids, saponins, terpenoids, phenols, glycosides, some metals and essential minerals. However, contamination of these plants with metals such as Pb and Cd can lead to the significant health risks to consumers such as indigestion, neurological disorders & carcinogenesis. This research underscore the significance of qualitative phytochemical screening and metal analysis in medicinal plants and its relevance in ensuring the safety & efficacy of herbal remedies. The findings generally indicates differences in the phytochemical and mineral level in plant. Thus, as a result this study has demonstrated the validation of these medicinal plants with respect to the presence of phytochemical and accumulation of heavy metals or minerals. Further research directions are proposed to enhance the formulation of potential herbal drugs and establish comprehensive regulatory frameworks for herbal products.

1. Introduction

The phytochemicals are the most important sources for the treatment of common diseases[26]. These chemical compounds are naturally found in plants like steroids, terpenoids, flavonoids, and alkaloids [16, 18]. According to World Health Organisation (WHO), medicinal plants would be the best source to obtain variety of drugs [17,19]. Qualitative phytochemical analysis will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings[8,16].

The metal analysis in plants is crucial and plays a significant role in identifying deficiencies or toxic level of metals in plants. Lead (Pb) and Cadmium (Cd) are examples of heavy metals that are not essential for plants, as they do not contribute to any known physiological functions[24]. Other metals such as Co, Cu, Zn & K are essential elements required for normal growth & metabolism of plants[1,14]. These elements can easily lead to poisoning when their concentration rises to supra-optimal value [2]. Moreover, medicinal herbs may be contaminated during growing & processing stages. The environment, pollution, the atmosphere, soil, harvesting and handling are some of the factors which could play an important role leading to the contamination of medicinal plants by metals[3,7]. The presents study was carried out on three medicinal plants (Entada rheedei, Tridax procumbens & Balanites aegyptiaca). These plants are used in the treatment, prevention and management of diseases. The Entada rheedei (Leguminosae) is used to induce vivid dreams so it is also known as African dream herb[4]. The seeds of this plants are used against diarrhea & stomach ache[5, 12] . These seeds are also known in the Egyptian herbal market as antirhematic, anti-inflammatory, dietary supplements and in the weight gain preparations [13]. T. procumbens (Asteraceae) is a wild herb distributed through the India, Nepal, and Nigeria is used in the treatment of bronchial catarrh, dysentery, diarrhea, and inflammation [1,6]. It originated in Mexico, South America, and Central America, although it may also be found in Tropical America, Africa, and Asia.[20]. In Ayurveda, T. procumbens is used as an herbal drug for wound cure. In India's tribal and rural people, this plant has been used as folk medicine and as a nutritional vegetable. The extracts of Tridax

procumbens have been reported to have various pharmacological effects like mosquito repellent activity, leishmanicidal, hepatoprotective effect on the liver antioxidant system, immunomodulatory effect; wound healing activity, and antiprotozoal effects[21]. Fresh leaves juice of T. procumbens has been used from ancient times to treat wounds, skin diseases, typhoid fever, fever, cough and to stop blood clotting in folk medicine in Africa[1,7]. Indigenous methods for the treatments of various diseases using a different formulation of this plant were reported in Ayurvedic, Unani, such as it is dispensed as 'Bhringraj' for liver disorders[8], and preventing hair loss, hair growth[9]. In Guatemala T. procumbens is used in gastrointestinal disorders, stomach pain diarrhea[1,10] In America, this plant is extensively used in the treatment of cold, anemia hepatopathies, and inflammations[1,11]. Balanites aegyptiaca (Zygophyllaceae) is known as 'Desert date' which is traditionally used in treatment of various ailments like jaundice, intestinal worm infection, wounds, malaria, syphilis, epilepsy, dysentery, constipation, diarrhea, hemorrhoid, stomach ache, asthama and fever[11,14, 22].

These medicinal plants have been selected because they have many traditional medicinal uses and widely used by people in most communities. Many people in developing countries live in extreme poverty and suffer from various diseases. So, there is a need to explore herbal medicinal plants within these regions. The goal is to validate the traditional uses of these plants for medicinal purposes.

2. Material & Methods

Plant collection: The plants Entada rheedei & Tridax procumbens have been collected from Pune (Maharashtra, India) and the third one that is Balanites Aegyptiaca was collected from Otur village (Maharashtra, India). These plants sample were authenticated from Botanical Survey of India (BSI), Pune where voucher specimen kept.

2.1 Qualitative Phytochemical analysis

2.1.1. Extraction of plants

The fresh seed kernel of Entada rheedei, Fruits of Balanites aegyptiaca and Leaves of Tridax procumbens were washed with tap water and dried under shade. The seed kernels, fruits and leaves of these plants were crushed to coarse powder. These coarse powder (50g) were subjected to successive extraction from nonpolar to polar solvent by using Soxhlet apparatus. The crude extracts of these plants are then utilised for qualitative analysis.

2.1.2. Preliminary phytochemical analysis for the extracts was carried out by using standard procedures [16, 17]

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids[16].

H_2SO_4 test: Extracts were treated with few drops of H2SO4. Formation of orange colour indicates the presence of flavonoids [16]

Detection of steroids

2 mL of acetic anhydride was added to 5 mg of the extracts, each with 2 mL of H_2SO_4. The colour was changed from violet to blue green in some samples indicate that the presence of steroids[16].

Detection of Terpenoids

Salkowski's Test: 5 mg of extract mixed with 2 mL of chloroform and concentrated H_2SO_4 carefully added to form a layer. An appearance of reddish brown colour in the inner face was indicates the presence of terpenoids[16].

Detection of phenols

Ferric chloride test: 10 mg of extracts were treated with few drops ferric chloride solution. Formation of bluish black colour indicates that the presence of phenol[16].

Detection of Saponins:

About 0.5 mg of the extract was shaken with 5 mL distilled water. Formation of frothing (bubbles) shows the presence of saponins[16].

Detection of Tannins:
A small quantity was mixed with water and heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins[16].

Detection of Carbohydrate:
3 ml of extracts were added to 2 ml of Molisch's reagent and resulting mixture shaken. 2 mL of concentrated H2SO4 was poured carefully down the side of the test tube. Formation of red or dull violet color at the interphase of the two layers indicates the presence of carbohydrates[5,18].

Detection of protein:
Biuret test: to 0.5 mg of extract equal volume of 40% NaOH solution and 2 drops of 1 % $CuSO_4$ solution was added. The appearance of violet color indicates the presence of protein[12,16].

Detection of oils and Resins
Test solution was applied on filter paper. It develops a transpaprent appearance on the filter paper. It indicates the presence of oils and resins[16].

Detection of Glycosides

Kellar-Killani test: 2 mL of the filtrate was added with 1 mL of glacial acetic acid. Then 1 mL ferric chloride was added with 1 mL concentrated sulphuric acid. Green blue coloration of solution indicates the presence of glycosides[18].

2.1.3. Results:
The pharmacological effects of these three plants are attributed to their bioactive compounds. The presence of phytochemical compounds are shown in the table No. 3.

2.1.4. Table No. 3.

Sr. No.	**Phytochemical**	**Qualitative analysis**[18]		
		Entada rheedei	**Tridax procumbens**	**Balanites aegyptiaca**
1	Flavonoids	++	+++	+
2	Steroids	+	+++	+++
3	Terpenoids	+++	++	++
4	Phenols	++	++	++
5	Saponins	+++	++	++
6	Tannins	+	+	+
7	Carbohydrate	+	++	++
8	Protein	++	+	+
9	Oils and Resins	+++	+++	+++

10	Glycosides	++	+++	+++

+ indicates presence of phytochemicals, ++ shows moderate concentration, +++ shows high concentration, -- indicates absence of phytochemicals

2.2 Metal analysis of plant by Atomic Absorption Spectroscopy (AAS)

2.2.1 Chemicals

Atomic absorption spectroscopical analysis of these plants was conducted by using chemicals of analytical grade. The acids like nitric acid, hydrochloric acid were used. Deionized water was utilized for the acid digestion process.

2.2.2 Acid digestion & Atomic Absorption Spectrophotometer analysis

An acid digest of each plant species was prepared by oxidizing 5.0 g of powdered plant sample with Nitric and hydrochloric acid (1:3) mixture. 1 mL of each acid digest was further diluted to 50 mL due to corrosive nature of acid used. These aliquots of the mixture were used to estimate lead (Pb), Cadmium (Cd), Copper (Cu), Zinc (Zn) & Potassium (K) by the use of atomic absorption spectrophotometer (Model AA-6880F). The blank and working standard were initially run, followed by samples. Each sample was analyzed three times and the data was reported as the average of these analyses in ppm[3].

2.2.3. Results & Discussion:

From the analysis, lead (Pb) and Cadmium (Cd) were present in all three plants. Copper was found below the detection limit (<0.010 ppm). Potassium was found only in Entada rheedei while in other two plants, it was below detection limit. Zinc was also present in all three plants.

2.2.4 Tables:

Table 2: Concentration of metals in ppm

	Quantity of Metals (ppm)						
Name of plant	Pb	Cd	Cu	Zn	K	Hg	As
Entada rheedei	0.4769	0.0388	0.1790	2.0383	0.8085	0.861	0.017
Tridax Procumbens	0.5075	0.0481	0.1559	0.0381	0.9552	0.128	0.325
Balanites aegyptiaca	0.6601	0.0416	0.3996	0.9753	0.8613	0.765	0.072

Table 3: Comparison of Dietary intake of some heavy metal from vegetables with the recommended dietary allowance for 60 Kg adult, according to WHO [15].

Elements	Recommended dietary allowance
Pb	210 ppm
Cd	60 ppm
Cu	900-30000 ppm
Zn	15000 ppm
Hg	1 ppm
As	1 ppm

3. Conclusion

From this study, it has been concluded that the selected plants are the source of secondary metabolites like steroids, terpenoids, tannins, flavonoids, glycosides and saponins. As a result of the presence of secondary metabolites, these plants have traditional medicinal and therapeutic uses. Therefore, extracts of these plants could be the good source for the formulation of drugs.

The metal analysis conducted in this plant provides valuable insights into the absorption and retention of heavy metals by plants. Our findings underscore the importance of comprehending the process of metal absorption

and movement within plants, particularly in the context of toxicity of plants, environmental pollution and human health. In the metal analysis, metals namely lead (Pb), Cadmium (Cd), Zinc (Zn), copper (Cu), Potassium (K) , Mercury (Hg) & Aesenic (As) detected.

Furthermore, our study highlights the potential of certain plant species as bio-indicators or phyto-remediators for heavy metal-contaminated soils. By identifying plants with high metal accumulation capacities, we can develop strategies for remediation and mitigation of metal pollution in agricultural and natural ecosystems. However, it is important to recognize the limitations of our research, including the necessity for the additional investigation into the long-term effects of metal exposure on plant growth, development, and ecosystem dynamics. Additionally, more comprehensive studies that are needed to clarify the molecular mechanisms underlying metal uptake, transport, and detoxification in plants. The metal concentrations in Entada rheedei, Balanites aegyptiaca, and Tridax procumbens are below the limits suggested by the WHO, indicating that these plants are not poisonous or toxic.

Conflict of interest

The authors has no conflict of interest to disclose.

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References

1. H. Kupper and E. Andersen, Metallomics, 8(3), pp.269-285, 2016.
2. N. Rascio and F. Navari-Izzo, Plant science, 180(2), pp.169-181, 2011.
3. C. K. Yap, M. Fitri, Y. Mazyhar and S. G. Tan, Sains Malaysiana, 39(3), pp.347-352,
1. 2010.
4. R. R. Deokar, S. R. Kamble, S. R. Mane and S. R. Patil, World Journal of Pharmaceutical
2. Research, 6(17), pp.405-409, 2017.
5. S. Sugimoto, K. Matsunami, H. Otsuka, Journal of Natural Medicines 66,
3. 552–557. doi:10.1007/s11418-011-0608-9, 2012
6. R. K. Verma and M. M. Gupta, Phytochemistry, 27(2), pp.459-463.
4. https://doi.org/10.1016/0031-9422(88)83120-0, 1988.
7. M. O. Soladoye, M. A. Sonibare, A. O. Nadi, D. A. Alabi, African J. Biotechnol. 4, 554 562. https://doi.org/10.5897/AJB2005.000-3102. 2005.
8. V. K. Saxena, S. Albert, J. Chem. Sci. https://doi.org/10.1007/BF02709296, 2005.
9. S. Saraf, A. K. Pathak, V. K. Dixit, Fitoterapia., 495-498, 1991.
10. M. H. Logan, Anthropos, (H. 3./4), pp.537-547, 1973.
11. A. Taddei, A. J. Rosas-Romero, https://doi.org/10.1016/S0944-7113(00)80009-4, 2000.
12. M. M. Okba, S. A. El Awdan, M. F. Yousif, K. S. El Deeb and F. M. Soliman, Journal of Applied Pharmaceutical Science, 8(5), pp.101-108, 2018.
13. M. M. Okba, F.M. Soliman, K. Deeb and Y. MF, International Journal of Pharmacy and Pharmaceutical Sciences, 5(3), pp.311-329, 2013.
14. D. L. Chothani and H. U. Vaghasiya, Pharmacognosy reviews, 5(9), p.55, 2011.
15. D. Demirezen and A. Aksoy, Journal of food quality, 29(3), pp.252-265, 2006.
16. K. Santhi and R. Sengottuvel, International Journal of Current Microbiology and Applied Sciences, 5(1), pp.633-640, 2016.
17. F. Mumtaz, S. M. Raza, Z. Ahmad, A. Iftikhar and M. Hussain, Journal of Pharmacy and Alternative Medicine, 3(3), pp.5-10, 2014.
18. R. Yadav, R. K. Khare and A. Singhal, Int. J. Life. Sci. Scienti. Res, 3(1), pp.844-847, 2017.
19. S. Arunkumar and M. Muthuselvam, World journal of agricultural sciences, 5(5), pp.572-576., 2009.
20. V. V. Ingole, P. C. Mhaske and S. R. Katade, Phytomedicine Plus, 2(1), p.100199, 2022.
21. S. L.Udupa, A. L. Udupa, D. R. Kulkarni, Planta Medica. **57**: 325-327, 1991.
22. J. B. Hall, D. H. Waljer, A monograph; pp. 1–12,1991.