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Original Article

A Comparative Chemical Profiling Between *Boerhavia diffusa* and its Adulterant *Trianthema portulacastrum* Herbal Plants

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Abstract

Boerhavia diffusa has many uses in the Siddha system of medicine and is misidentified sometimes as *Trianthema portulacastrum* L., due to its close resemblance. This specific study compares, evaluates and identifies the chemical constituents profiling of the two whole plant drugs based on inorganic materials determination, various phytochemical aspects and advanced chromatographical studies**.** The studied plants *i.e*., *Boerhavia diffusa* and *Trianthema portulacastrum* showed the peculiar characteristics and having significant differences in these chemical profiling. *Boerhavia diffusa* had higher levels of the primary and secondary metabolites than *Trianthema portulacastrum*. *Boerhavia diffusa* differs from *Trianthema portulacastrum* in distinct ways. The pH is differed in both plants *viz.* 6.45 in *B. diffusa* and 5.2 in *T. portulacastrum*. The total ash, water soluble ash and acid insoluble ash is different in both the plants. *B. diffusa* and *T. portulacastrum* have alkaloids 628.00 and 1417.00 (mg/100g), Terpenoids 146.67 and 174.46 (mg/100g), flavonoids 223.00 and 846.00 (mg/100g), tannins 30.50 and 32.67 (mg/100g), phenols 28.40 and 74.80 (GAE/g), carotenoids 248.00 and 968.00 (mg/100g), oxalate 151.00 and 143.83 (mg/100g) respectively. *diffusa*. HPTLC fingerprint profile of the *B. diffusa* showed 8 peaks among them the peak at R_f 0.12 is the major peak, however *T. portulacastrum* showed 6 peaks among them the peak at R_f 0.27 is the major peak.

Keywords**:** *Adulterant; Antioxidants; Boerhavia diffusa; Herbal Medicines; Trianthema portulacastrum*

Introduction

Plants are main sources for human health care. The plants have specific active constituents which are useful in various ailments (Kashyap, 2002). Recently the awareness about herbal plants importance is at high level. Natural plants and essential oils have the potential in the prevention and treatment of poultry diseases also (Cross *et. al.*, 2007, Ekunseitan *et. al.*, 2017). Since the dawn of civilization, medicinal plants have been highly valued for healthcare. Their habitats, influenced by various environmental factors, can produce potentially harmful secondary metabolites. All parts of these plants are used for medicinal purposes, and they have been administered for diverse diseases for a long time (Ali *et al.*, 2022). The secondary metabolites play an important role to control the metabolic activities in the animals and human body (Ekunseitan *et. al.*, 2016). They have potential character to treat the animals. *Boerhavia diffusa* has been used in healthcare since traditional knowledge was first incorporated into our civilization. The plant is renowned for its rejuvenating properties (Ali *et. al.*, 2022). Many herbal companies and pharmaceutical units are using large quantities of *Boerhavia* crude drugs. *Boerhavia diffusa*, specifically, has demonstrated antidiabetic properties (Ali *et. al.*, 2022).

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Medicinal plants have peculiarities in secondary metabolism and in composition of their unique biologically active substances. The medicinal properties of natural herb (Gardzielewska *et. al.*, 2003) need to be explored. Traditional herbal medicines currently help to improve health care and, therefore, the majority of the world's population is moving towards them and this may increase in the future. The overarching goal of drug development is quality, safety, and efficacy.

B. diffusa has several medicinal benefits like it helps for male/female reproductive disorders (roots) (Upadhyay *et. al.*, 2007), act as antihepatotoxic (Kaur, 2019) and diuretic (Pandey & Shukla, 2008).

In the modern time the herbs are highly exploited for the crude drugs processing but there is a fair chance to adulterate the crude drugs. *Boerhavia diffusa* L. and *Trianthema portulacastrum L.* are widespread throughout India. *B. diffusa* is a perennial diffuse herb Quality Standard of Indian Medicinal Plants, ICMR, (2011) whereas *T. portulacastrum* is a prostrate glabrous annual succulent herb Quality Standard of Indian Medicinal Plants, ICMR (2005). The former finds its widespread use in the Siddha system of medicine, but because of the similar resemblance it is often confused with the latter and therefore often frequently contaminated or adulterated with *T. portulacastrum*.

The use of a wrong herb as medicine may result as ineffective or mild effective treatment. Due to the global popularity of herbal drugs and their consequent demand is increasing day by day. Ultimately imbalanced demand promotes the adulteration or alternative sources uses in supply. This practice may be either intentionally or unknowingly because these herbal plants are supplied by traders to the pharmacies. Sometimes the true medicinal plant is replaced by traders who have no proper knowledge about the particular herbs. The reasons behind this adulteration includes economic pressures, profit maximization, consumer ignorance, regulatory deficits, sophisticated and less detectable adulteration techniques.

Both traditional methods of analysis, such as TLC, DNA barcoding, and phytochemical screening, and advanced analytical techniques like HPLC, GC, and NMR spectroscopy are used for the authentication and identification of medicinal plants (Devi, 2025). Kumar *et al*. (2025) and newer tools, such as AI-based systems, focus on accurately measuring bioactive compounds. This ensures consistency in batches for authentication and classification (Kaur *et al*., 2025). This study compares, analyzes, and identifies the chemical constituents of two whole plant drugs. It is based on the determination of inorganic materials, various phytochemical aspects, and advanced chromatographic studies. The plants studied, Boerhavia diffusa and *Trianthema portulacastrum*, exhibited distinct characteristics and significant differences in their chemical profiles.

This study will help in finding distinguishing characters to obtain a clear picture on differentiating the two crude drugs by chemical profiling and pharmacognostical characteristics, when traded in the market as Mukkirattai.

Materials and Methods

Boerhavia diffusa were collected from local wild area of Modinagar, District Ghaziabad and identified from the Herbarium of Multanimal Modi College, Modinagar and material was compared with preserved herbarium.

Inorganic Materials Determination

The determination of inorganic materials, such as Total ash, water-soluble, acid insoluble ash and pH determination were carried out. For ash determination the dried material of 5 g each of *Boerhavia diffusa* and *Trianthema portulacastrum* have been taken and ignited at 450° C until the white colored ash**.** It was carbon free ash. This white ash is added in HCl (6N) and heated for 5 minutes to separate the insoluble material. Remaining water-soluble material is filtered by filter paper. Later on, it is dried at 450° C to dry the ash. It is kept for 6 hours at 250° C and dried the ash. This dried ash is stored for further testing.

Phytochemical screening

Different types of phytochemical constituents and active constituents are found in the studied plants.

Active Constituents

Every plant has its peculiar character in the form of active constituents which are developed just after secondary metabolism. The secondary metabolites may be identified by the peculiar character of fragrance, flavor, dyes or pigments etc. These are useful in the ailment of different diseases. These chemical compounds have medicinal importance in the development of the drugs. Active constituents are natural products and are also called secondary metabolites. They are used in many industries for the development of drugs, flavor, pesticides and food additives. The demand of these active constituents increases commercially day by day. Secondary metabolites like alkaloids, flavonoids, terpenoids, carotenoids, tannins, etc. are obtained by various extraction methods. The determination of alkaloids and flavonoids done according to the Li *et. al*. (2015), Harborne (1998) and Mujeeb *et. al.*, (2014). The determination of tannins done according to the Mujeeb *et. al.*, (2014). The determination of phenols done according to the Obodoni and Ochuko (2001) and Mujeeb *et. al*., (2014). The determination of carotenoids done according to the Butnariu (2016). The determination of terpenoids done according to the Indumathi *et. al*. (2014). The determination of oxalate done according to the Liu *et. al*. (2015).

Chromatograms

The studies were carried out using High-Performance Thin-Layer Chromatography (HPTLC) with the assistance of CAMAG instrumentation. Both the plants fingerprints of the methanolic extracts of *Boerhavia diffusa* and *Trianthema portulacastrum* were used, reconstituted with methanol and developed TLC. The samples were used at silica gel thin layer (60 F_{254} plates). It has 0.20 mm thick layer and scanning were made at the 450 nm visual range in solvent system and finally scanned at 254 nm wavelengths. The best fingerprint was reported in *Boerahavia diffusa* sample. It was applied on precoated silica gel 60 F_{254} TLC plates (5 X 10 cm) by Linomat 5 sample applicator. The sample was applied triplicate 8 µl each, the width of the track was 5 mm and distance between tracks was 13 mm. There after the plate was scanned at 254 nm and after spray with anisaldehyde sulphuric acid at suitable wave length. The plate was then spayed with anisaldehyde sulphuric acid reagent and kept in oven followed by air drying. The chromatograms were scanned at visible range 450 nm and results were observed. For the HPTLC fingerprinting of different sample extracts first of all a suitable solvent system was developed and prepared a TLC.

Chromatographic Conditions of HPTLC:

Parameter	Condition
Sample	Extract 20mg/ml dissolved in methanol
TLC Plate	Pre-coated silica gel 60 F254 plates (E. Merck, 0.20 mm thickness)
Solvent System	Toluene:ethyl acetate:formic acid (5:4:1, v/v/v)
Detection	UV 254nm and at 450 nm sprayed by anisaldehyde sulphuric acid
Scanner	Reprostar scanner 3

Results

Inorganic Materials Determination

In the present study both the plants showed a significant difference in inorganic materials like total Ash value, water soluble ash, acid insoluble ash, pH and foreign materials (Table 1).

Table 1: *Comparision of Finding Values with IPC of B. diffusa & T. Portulacastrum*

Parameters	Boerahavia Diffusa		Trianthema Portulacastrum
	Values In Ipc*	Finding Values	Finding Values
Total Ash values	15%	4.5 %	3.87%
Water soluble Ash	4%	1.3%	1.66%
Acid Insoluble Ash	6%	2.13 %	2.63%
Weight loss on drying	8.0%	8.0%	6.83%
Foreign material	≤ 2%	≤ 0.46%	≤ 0.14%
Matter pH	≤ 6.5	≤ 6.45	≤ 5.20

IPC= Indian Pharmacopoeia Commission*

The pH is differed in both plants *viz.* 6.45 in *B. diffusa* and 5.2 in *T. portulacastrum*. The ash value and water solubility are different in both the plants.

Quantitative Analysis

During the phytochemical screening, the plant materials were soaked in the extraction medium for 1-4 hour with continuous stirring and later on cooled it at room temperature and filtered. The process was repeated again and again and the solvents were recovered by distillation and phytochemical screening were made which are shown in table-2.

Table 2: *Solubility of B. Diffusa and T. Portulacastrum in Percent of Their Dry Weight in Different Solvent*

Parameters	Plant	
	B. diffusa	***T. portulacastrum***
Solvent: Petroleum Ether	23.67%	20.99%
Solvent: Chloroform	11.12%	10.61%
Solvent: Alcohol	5.10%	11.09%
Solvent: Distilled Water	1.9%	3.1%

The results of secondary metabolites showed the significant changes in between the studied plants. During the phytochemical screening, the plant materials were processed and kept it at room temperature. The secondary metabolites in whole plant were scrutinized as alkaloids, terpenoids, flavonoids, tannins, phenols, carotenoids, and oxalate shown in table-3.

Table 3: *Phytochemical Analysis of Boerhavia Diffusa and Trianthema Portulacastrum*

Parameters	Plant	
	B. diffusa	***T. portulacastrum***
Alkaloids (mg/100g)	628.00b ±0.58	1417.00a ±0.58
Terpenoids (mg/100g)	146.67b ±0.89	174.46a ±0.58
Flavonoids (mg/100g	223.00b ±0.58	468.00a ±0.58
Tannin (mg/100g)	30.50b ±0.29	32.67a ±1.2
Phenols (GAE/g)	28.40b ±0.31	74.80a ±0.58
Carotenoids (mg/100g)	248.00b ±0.58	968.00a ±0.58
Oxalate (mg/100g)	151.00b ±0.58	143.83a ±0.89

The superscripts 'a' and 'b' denote statistically significant difference in the mean values of 5% significance level (P<0.05).

T. portulacastrum have qualitatively more amount in comparison to *B. diffusa*. *B. diffusa* and *T. portulacastrum* have alkaloids 628.00 and 1417.00 (mg/100g), Terpenoids 146.67 and 174.46 (mg/100g), flavonoids 223.00 and 846.00 (mg/100g), tannins 30.50 and 32.67 (mg/100g), phenols 28.40 and 74.80 (GAE/g), carotenoids 248.00 and 968.00 (mg/100g), oxalate 151.00 and 143.83 (mg/100g) respectively.

Chromatographically analysis Results

Chromatographical studies were also made with the help of HPTLC. It was found that maximum peak in *B. diffusa* is 500 AU (0.12 R_f value) while in case of *T. portulacastrum* it determined at nearby 400 AU (+/- 0.30 R_f). Good chromatogram of *Trianthema portulacastrum* was observed at 455 nm after sprayed with anisaldehyde-sulphuric acid reagent. The Rf values of both the plant sample given in the table-4 and proline profile in table 5.

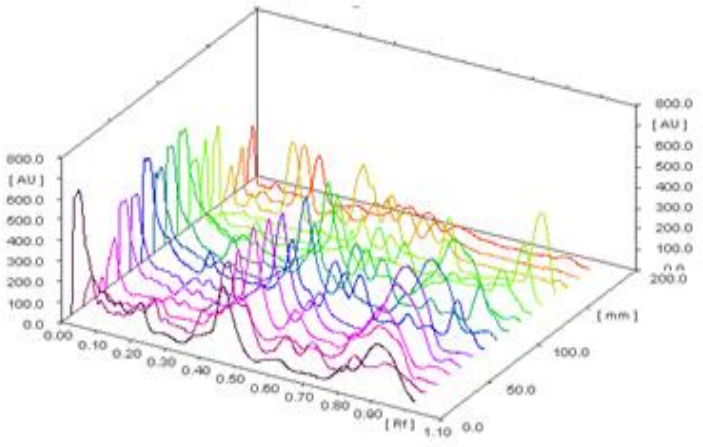
Table 4: *R_f Values of the Boerhavia Diffusa and Trianthema Portulacastrum Plant Samples*

Sample	**No of spots and Rf values**
Boerhavia diffusa	(8) 0.09, 0.12, 0.19, 0.27, 0.35, 0.62, 0.68, 0.72
Trianthema portulacastrum	(6) 0.27, 0.42, 0.60, 1.18, 1.32, 1.67

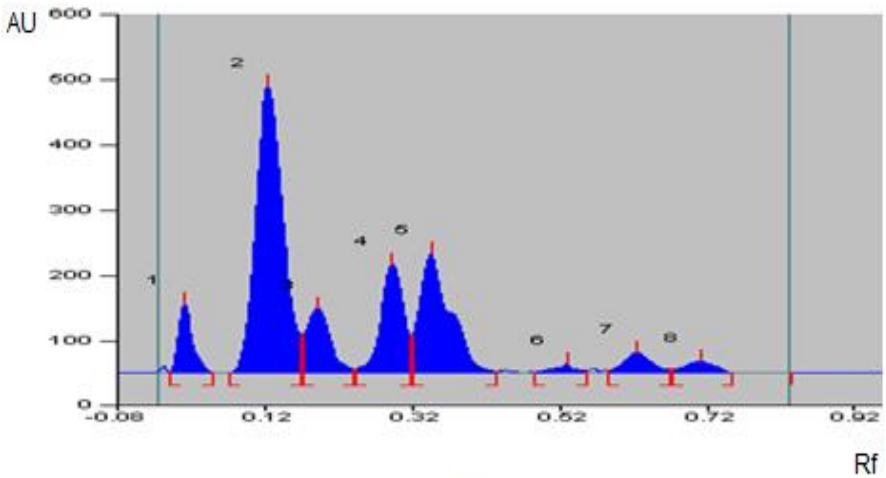
Table 5: *Proline Qualitative Tests Different Seasons*

SN	Plants	Site A (Near River)	Site B (Near Field)	Site C (Near Road)	Site D (Rocks)
1.	*B, diffusa*	++	++	++++	+++
2.	*T. portulacastrum*	+++	+++	+++++	++++

Note: + =low reaction, ++ =moderate reaction, +++ =high reaction, - =no reaction



A



B

Figure 1: *HPTLC fingerprint profile of Boerhavia diffusa (A & B)*

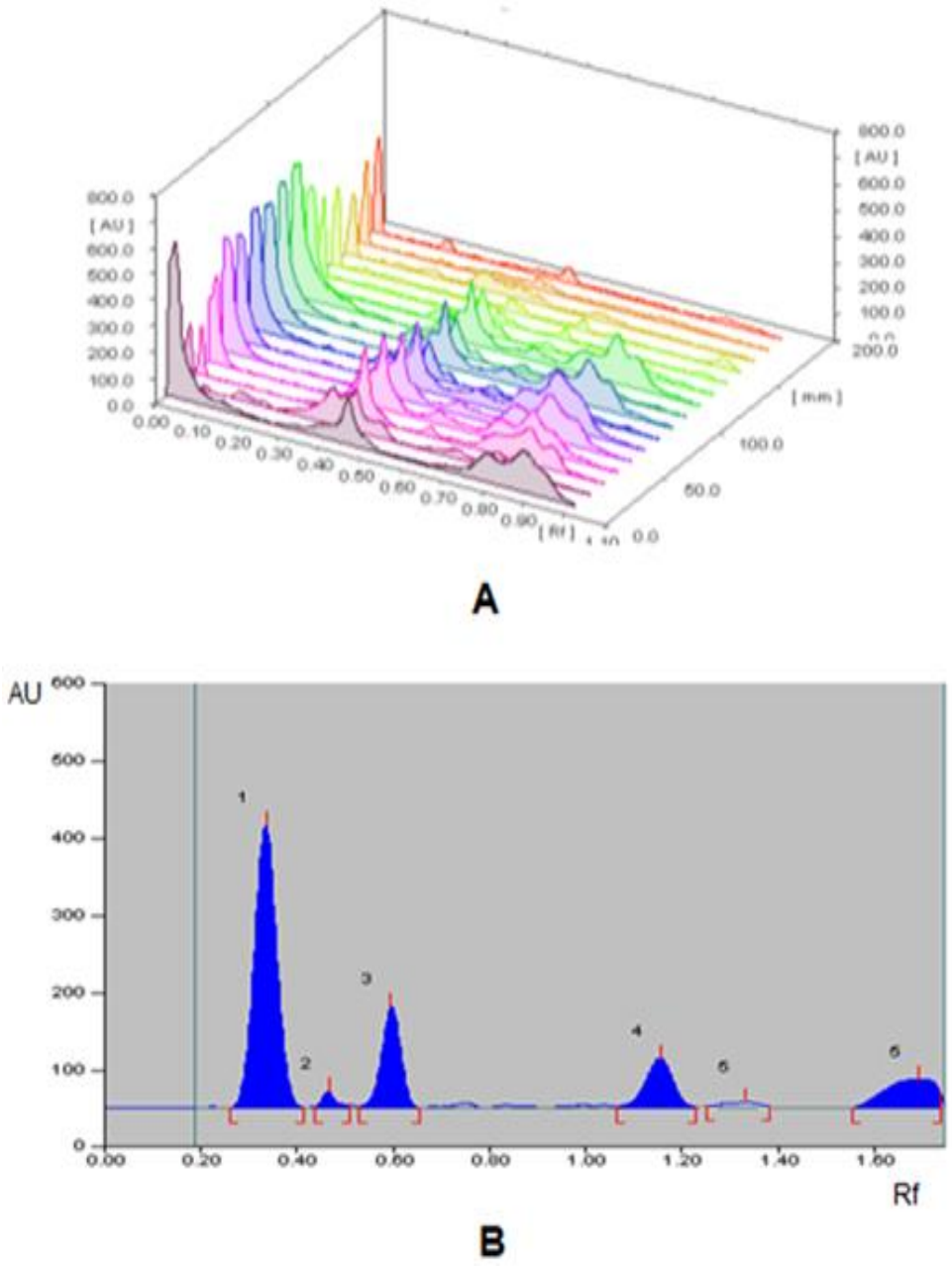


Figure 2: *HPTLC fingerprint profile of Trianthema portulacastrum (A & B)*

HPTLC fingerprint profile of *B. diffusa* showed 8 peaks at R_f 0.09, 0.12, 0.19, 0.27, 0.35, 0.62, 0.68, 0.72, among them the peak at R_f 0.12 is the major peak. However, in *T. portulacastrum* showed 6 peaks at R_f 0.27, 0.42, 0.60, 1.18, 1.32, 1.67, among them the peak at R_f 0.27 is the major peak.

Discussion

In the present study both the plants showed a significant difference in quantitative analysis of secondary metabolites, and other inorganic materials. In similar studies, Adeyemi *et al*. (2017) conducted a phytochemical analysis and GC-MS profiling of *Boerhavia diffusa R.* fruit, and that findings support the present study. Ekunseitan (2016) carried out a comparative analysis of two plants, *Boerhavia diffusa* and *Petiveria alliacea,* reinforcing the relevance of this work. Ali *et al*. (2022) investigated storage specificity in *Boerhavia diffusa* Linn as a crude drug and observed significant changes in active constituents over time. Likewise, Kashyap *et al*. (2007) performed comparative studies on habitat and storage specificity for various medicinal plants. More recently Mutha *et al*. (2025) applied modern

analytical techniques to study storage specificity of *Adhatoda zeylanica* as a crude drug, employing sophisticated methods for quality control and chemical identification of phytochemicals. Gupta (2002) did the pharmacognostical studies of *Grewia* sp. and observed the different elements of the medicinal plants like the present studies. Balkrishna *et. al.* (2025) worked on the traditional medicinal plants for gastrointestinal and hepato-renal disorders in the Ganga River watershed. Gupta and Jain (1979) worked on the estimation of total alkaloids of *Adhatoda vasica* Nees and found different types of alkaloids at different stages which supports the present studies. In the present study, the HPTLC shows the significant changes between the studied plants. Singh *et. al*., (2019) also did the same work on estimation of boeravinone-b by HPTLC Chromatograms in *Boerhavia diffusa L.* and its polyherbal dosage form are clearly different.

Limitation and Future Prospects

The present study holds great potential for future research to develop standardized protocols, harmonized QC guidelines for identifying genuine medicinal plants and quick testing methods to ensure the quality and effectiveness of herbal products. This can improve manufacturing and trading practices, benefiting both pharmaceutical companies and consumers. Strengthening international regulations, quality control, monitoring of drug safety, collaboration across fields, training, and consumer education will be essential for controlling adulteration and protecting the safety and trustworthiness of herbal medicine.

Conclusion

The chemical profiling of *Boerhavia diffusa* Linn had higher level of phytochemical constituents than its adulterated plant *Trianthema portulacastrum*. Viz. The total alkaloids in *B. diffusa* were 6.45 but in *T. portulacastrum* it was 5.2. The total alkaloids in *B. diffusa* were 628 and 1417 (mg/100g). The terpenoides 146.67 in *B. diffusa* while 174.46 (mg/ 100g) in *T. portulacastrum*. Flavonoids in *B. diffusa* was 223 but it was seen very high in the *Trianthema* 468.00 mg/ 100gms. Tannin was 30.50 mg/100gms in *B. diffusa* while it was 32.67 mg/ 100gm in *Trianthema*. Phenols were 28.40 in *B.diffusa* but in *T. portulacastrum* it was found 74.80 mg/100gm. The Oxalate was 151.00 in *B. diffusa* and in *T. portulacastrum* it was found 143.83 mg/ 100gm. HPTLC profiling showed eight peaks for *B. diffusa* with a major peak at Rf 0.12, whereas T. *portulacastrum* had a major peak at Rf 0.27, establishing distinctive chromatographic differences. These chemical and chromatographic distinctions serve as diagnostic tools to differentiate the true medicinal plant from its adulterant, thereby preventing adulteration in herbal medicine preparation. This work not only advances scientific understanding of traditional herbal medicine but also lays a foundation for integrating such knowledge into modern healthcare practices.

Conflict of Interest

The authors declare that they have no competing interests.

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