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Powder Microscopy, Fluorescence, Qualitative Phytochemistry, and GC- MS analysis of a Neglected Ethnomedicinal Weed - *Ruellia brittoniana* Leonard

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Abstract

Ruellia brittoniana is a common wild ethnomedicinal plant belonging to the Acanthaceae family. The plant was claimed ethnically wound-healing properties, use as an antidote against snake bites and to cure bone fractures. However, these claims are not been validated. The present study deals with powder microscopy, fluorescence, and qualitative phytochemical analysis of the leaves, stem, and root of this plant. The powdered microscopy showed the presence of both types of trichomes and spiral elements. The Fluorescence analysis under visible and ultra-violet light for leaves, stems and root powder treated with various chemical reagents revealed different patterns of fluorescence effect. The qualitative phytochemical study showed that the plant is rich in phytochemicals and possesses significant levels of Glycosides, alkaloids, flavonoids, terpenoids, steroids, and saponins. The availability of these groups of Phyto- components indicates that the plant could prove an alternative remedy to cure asthma, fever, bronchitis, high blood pressure, eczema, and diabetes. Further, the GC-MS analysis revealed the 10 phytochemical compounds have significant medicinal potential. The major objective of this study is to endorse this plant as a step toward commercial drug developments after confirming and standardizing its microscopic features and fluorescence behavior to identify adulteration in market-available crude drug powder and screening for possible drug molecules.

Keywords: *Ruellia brittoniana*, powder microscopy, fluorescence, phytochemistry, GC-MS.

INTRODUCTION

Since civilization, humans are using plants and plant products for their various needs including routine healthcare. As plants possess healing properties, they are being used as traditional medicinal plants across the globe, especially by different tribes. Now, it's a known fact that the properties of medicinal plants are due to their active secondary metabolites. The actual herbal era started after isolating active compounds like digitoxin, quinine, cocaine, and codeine from different medicinal plants (Wachtel-Galor *et al*, 2011).

The genus *Ruellia brittoniana* Leonard belongs to the family Acanthaceae (Acanthus family). It is native to Mexico (Mexican petunia), The Caribbean, and South America. Later in the early 20th Century it was introduced in various European and Asian countries and become invasive. It is an evergreen perennial plant (Elgindi *et al*, 2015). The plant growing in its actual habitat and its morphological details are given in fig. 1A, B & C. The present study is focused on powder microscopy, fluorescence, and qualitative phytochemical analysis of the plant *Ruellia brittoniana* and GC-MS analysis of its ethanolic leaf extract.

Figure 1: A) Photographs from the natural habitat of *Ruellia brittoniana* B) & C) Photographs showing flowers and pods of *Ruellia brittoniana* respectively

MATERIAL AND METHODS

The plant was collected from various places in the Western Vidarbha region and identified using the flora of Marathwada (Naik, 1998) and the flora of Maharashtra (Singh and Karthikeyan, 2000). The collected plants after identification, were dried under shade for about 7- 10 days and then ground into fine powdered with the help of a blender.

Powder Microscopy

The powdered material of leaves, stem, and root of *Ruellia brittoniana* was soaked in 20% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked spice powders with safranin and observed under the microscope, and the images were captured (Carl Zeiss Binocular Research Microscope, PRIMOSTAR). The powdered microscopy was done as per Kokate *et al*, (2004); (API, 2007), and Hole *et al*, (2008).

Fluorescence Analysis

The coarsely powdered samples of leaves, stem & root (1 g each) were treated separately with different reagents (5ml each) such as acids and alkaline solutions along with other solvents inside clean test tubes and shaken well and allowed to stand overnight. The individual solutions were observed under visible light and UV light for their characteristic color and compared with a standard color chart (Shirsat and Suradkar, 2017; Jasutkar *et al*, 2018) and all the observations were taken as per Aeri *et al*, (2020).

Qualitative Phytochemical Analysis

For qualitative phytochemical analysis, standard protocols were followed (Harborne, 1998; Krishnaiah *et al*, 2009; Koche *et al*, 2010). The reagents and chemicals used were of SD Fine and QualiChem make.

GC- MS analysis

The GC-MS analysis of these extracts was availed from SAIF, CDRI, Lucknow that performed with GC Clarus 500 Perkin Elmer system and GC interfaced to an MS equipped with an Elite-1 fused silica capillary column (30 mm × 0.25 mm 1D × 1 µm of the capillary column, composed of 100% Dimethylpolysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (Split ratio of 10:1); Injector temperature 250°C; ion-source temperature 280°C.

The oven temperature was programmed from 110°C (isothermal for 2 minutes) with an increase of 10°C/minute to 200°C, then 5°C/minute to 280°C, ending with 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV, a scanning interval of 0.5 seconds, and fragments from 45 to 450 kDa. The total GC running time was 36 minutes. The plant extract was dissolved in ethanol and filtered with a polymeric solid phase extraction column and analysed in GC-MS for different components. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra, and chromatograms were a turbo mass. Interpretation of the mass spectrum of GC-MS was done using the database of the National Institute of Standards and Technology (NIST).

RESULT AND DISCUSSION

The detailed observations on powder microscopy, fluorescent analysis, qualitative phytochemical analysis, and GC-MS analysis of methanolic extracts of *Ruellia brittoniana* are presented in the following paragraphs.

Powder Microscopy

Leaves: The powder is fine and moss green in color without any taste or odor. It showed the presence of epidermal cells with stomata, unicellular as well as multicellular trichomes, thick fiber walls, parenchymatous cells, and chlorophyll tissues. The powder also showed the presence of many square and prismatic crystals of calcium oxalate shown in Fig. 2 A, C, D and E.

Stem: The powder of the stem is yellowish-green in color without any odor or taste. It showed the presence of multicellular uniseriate trichomes, a group of parenchymatous cells, conducting elements, tissue fibers and vascular tissues with vessels of fibers, fragmented fibers, fragments of vessel elements bearing spiral pitted thickening shown in Fig. 2 A, E, G, and H.

Root: The powder of root is fine and greyish brown or rusty brown in color without any taste or odor. It showed spiral vessel elements, vascular tissues, fragmented fibers, parenchymatous cells, epidermal cells, irregular brown cork cells, aseptate fibers with tapering ends, and broken aggregated calcium oxalate crystals. Root hairs, mesocarp cells, etc. shown in fig. 2. A, B, F, and G.

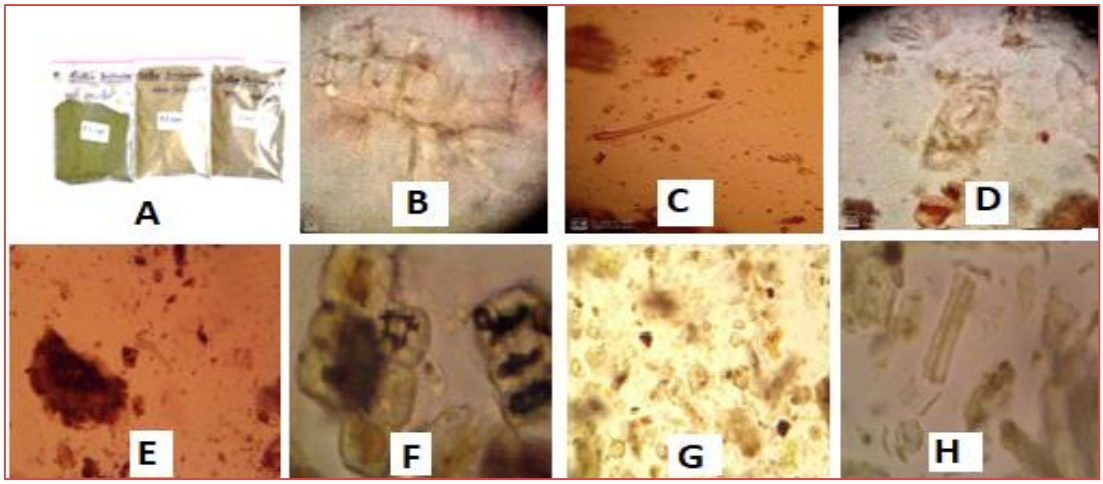

Figure 2: Images of powder microscopy

A) Power of leaf, stem & root of *Ruellia brittoniana* B) Epidermal cells C) Unicellular trichome D) Stomata E) Multicellular trichome F) Vascular tissue G) Calcium oxalate crystals H) Conducting elements.

Fluorescence Analysis

The powdered material of shade-dried leaves, stem, and root of *Ruellia brittoniana* was analyzed for its physical properties, especially its coloration; untreated as control and after the addition of various routine laboratory chemicals under visible light as well as UV light. The results showed that there is a drastic color change when the reaction mixture was exposed to both visible light and UV light. It is an important criterion to decide the authenticity of the market available crude powdered drugs of traditional medicinal plants. The fluorescence characters of leaf, stem, and root powders of *R. brittoniana* are noted in Table 1, Table 2, and Table 3. It was noted that with the same reagent, the powder sample showed different coloration in visible and UV- light. Also with different reagents, different colorations were seen (Fig. 3. A, B, C, D, E, F). This might be the marker test for the authenticity of the crude powder drug of the plant.

Table 1: Fluorescence Analysis of *R. brittoniana* Leaf Powder

Sr. No.	Chemicals	Visible light	UV light
1.	H_2SO4	Black	Black
2.	50% H_2SO4	Black	Black
3.	HNO_3	Yellow	Moss Green
4.	50% HNO_3	Orange Yellow	Blackish Green
5.	1N HCL	Greyish Brown	Grey
6.	Acetic Acid	Blackish Green	Pinkish
7.	1N NaOH	Brown	Dark brown
8.	1N NaOH+ Methanol	Green	Pinkish
9.	50% KOH	Brown	Blackish
10.	Ammonia	Yellowish	Rusty Green
11	Chloroform	Leafy Green	Pink tinge

Table 2: Fluorescence Analysis of *R. brittoniana* Stem Powder

Sr. No	Chemicals	Visible light	UV light
1.	H_2SO4	Black	Black
2.	50% H_2SO4	Black	Black
3.	HNO_3	Yellow	Moss Green
4.	50% HNO_3	Light Green	Moss Green
5.	1N HCL	Brownish	Pinkish tinge
6.	Acetic Acid	Greyish Yellow	Colourless
7.	1N NaOH	Orange Brown	Brown
8.	1N NaOH+ Methanol	Yellowish Brown	Brownish Green
9.	50% KOH	Orange Brown	Blackish Green
10.	Ammonia	Brownish	Moss Green
11	Chloroform	Colourless	Pinkish

Table 3: Fluorescence Analysis of *R. brittoniana* Root Powder

Sr. No	Chemicals	Visible light	UV light
1.	H_2SO4	Black	Black
2.	50% H_2SO4	Black	Black
3.	HNO_3	Yellow	Moss Green
4.	50% HNO_3	Light Green	Moss Green
5.	1N HCL	Light Brownish	Greyish Pink
6.	Acetic Acid	Brownish	Pinkish
7.	1N NaOH	Orange Brown	Brownish Green
8.	1N NaOH+ Methanol	Orange Brown	Moss Green
9.	50% KOH	Orange Brown	Blackish Brown
10.	Ammonia	Brown	Black
11	Chloroform	Colourless	Colourless

There are few reports on powder microscopy of some medicinal plants like that *Ocimum gratissimum* (Gupta *et al,* 2011); *Hilleria latifolia* (Amponsah *et al,* 2014); *Vitex negundo* (Bharathi *et al,* 2017) and *Colebrookea oppositifolia* (Shirsat and Suradkar, 2017) indicating the importance of work to authenticate the market available crude drug. Our work is an addition to the standardization of powdered drug material.

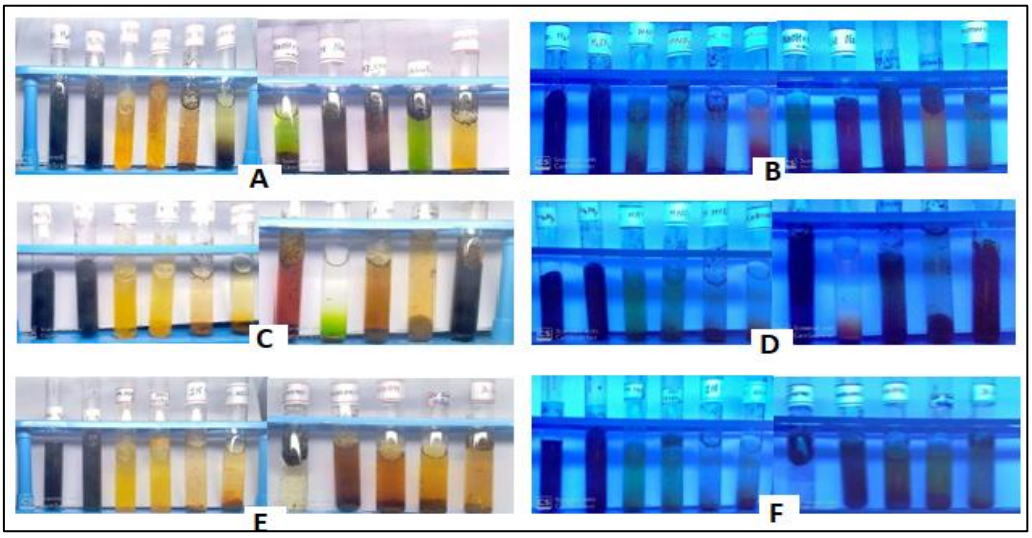

Figure 3: Images showing fluorescence colors

A) & B) Leaf of *R. brittoniana* fluorescence at visible and UV light respectively,
C) & D) Stem of *R. brittoniana* fluorescence at visible and UV light respectively,
E) & F) Root of *R. brittoniana* fluorescence at visible and UV light respectively.

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of *Ruellia brittoniana* leaf, stem, and root extract showed that it is rich in phytoconstituents. The qualitative tests were done in three solvents i.e., methanol, acetone, and petroleum ether. Methanol was observed to be more useful the for extraction of phytochemicals than the rest of the solvents. The methanol extract showed the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenolics, tannins, terpenes, steroids, reducing sugar, and saponins. (Table-4). In Acetone extract carbohydrates, terpenes, steroids, and, reducing sugar were absent. While petroleum ether extract showed positive tests for alkaloids, carbohydrates, terpenes, steroids, reducing sugar saponins, and flavonoids, phenolics, were absent (Table-4). Further, the qualitative tests for most of the phytochemicals are found positive in roots powder followed by leaf powder and stem powder respectively. The tests for alkaloids, glycosides, saponins, reducing sugar and carbohydrates are positive in all powder samples. Phenolics, flavonoids, steroids, and terpenes are found absent in acetone and petroleum ether extracts of leaves, stem, and root powder of *R. brittoniana*. The results of the qualitative analysis of powder material are presented in table-4 and Fig. 4 A, B & C.

Phytochemicals are the biologically active compounds having medicinal properties. The qualitative phytochemistry of plants indicates the basic bioactive compounds present in that plants. Earlier, several workers had presented the phytochemical analysis of different medicinal plants. Gupta *et al,* (2013) revealed a qualitative phytochemical analysis of four different

medicinal plants. Angidew *et al*, (2022) reported a phytochemical analysis of some medicinal plants from Ethiopia. Ashwathi Shrinivasan & Angayarkanni (2023) reported qualitative phytochemicals from 17 different therapeutically important plants from diverse habitats. Qualitative phytochemical analysis of any plant gives an insight into the phytoconstituents present in that plant and possible medicinal properties possessed by the plant. It is an essential step in the phytochemical analysis of any given plant.

Figure 4: Qualitative phytochemical tests of *R. brittoniana* powder material
A. in methanolic extract, B. in acetone extract & C. in petroleum ether extract

Table 4: Qualitative Phytochemical Analysis of *Ruellia brittoniana* Leaf, stem, and root extract

Plant species	**Solvents or Extracts**	**Test for phytoconstituents**										
		Alkaloids	Glycosides	Phenolics	Flavonoids	Tannins	Terpenes	Steroids	Saponins	Red. Sugar	Carbohydrates	Proteins
Ruellia brittoniana Leaf	Me	+	+	+	+	+	+	+	+	+	+	-
	Ac	+	+	+	+	+	-	-	+	+	+	+
	PE	+	+	-	-	-	+	+	+	+	+	+
Ruellia brittonia na Stem	Me	+	+	+	+	+	+	+	+	+	+	-
	Ac	+	+	+	+	-	-	-	+	+	+	-
	PE	+	+	-	-	-	-	-	+	+	+	-
Ruellia brittonia na Root	Me	+	+	+	+	+	+	+	+	+	+	-
	Ac	+	+	+	+	+	+	+	+	+	+	-
	PE	+	+	-	-	-	-	-	+	+	+	-

NOTE: Me= Methanol extract; Ac= Acetone extract; PE = Petroleum ether extract

GC- MS analysis

The major compounds identified include- 3,7,11,15-tetramethyl-2-hexadecen-1-ol (28.75%) as the major component followed by α-sitosterol (15.35%), 9,12-octadecodienoic acid (10.27%), 1,1,3-triethoxy Propane, (8.21%), phytol (8.15%), α-amyrin (6.15%), squalene (5.75%), γ-tocopherol (3.85%), 9,12,15-octadecatrienal (2.39%), 1,2-benzene dicarboxylic acid di-heptyl ester (1.75%). Kartika et al., (2016) presented a similar GC- MS analysis in *Ruellia patula* from the South of India. Konappa *et al*, (2020) reported the GC- MS analysis of *Ammomum* species

with various phytochemicals. A similar report of GC MS analysis of Hibiscus asper was made by Olivia *et al*, (2021). This indicates that to identify the active compounds in plants, GC- MS plays a vital role. Our report is similar and made a significant contribution in identifying phytochemical compounds from *R. brittoniana*. This also helps to know the putative therapeutic potential of the identified phytochemicals and thereby the their source plant.

Table 5: GC- MS analysis of methanol extract of *R. brittoniana* leaves

Sr. No.	RT	Peak Area	MW	Molecular Formula	Compound identified
1	2.87	8.25	176	$C_9H_{20}O_3$	1,1-3 tri ethoxy propane
2	11.70	28.75	296	$C_{20}H_{40}O$	3,7,11,15- tetra-methoxy- 2hexadecen-l-ol
3	13.17	1.75	362	$C_{22}H_{34}O_4$	1-2 benzine-dicarboxylic acid di-heptyl ester
4	15.03	8.15	296	$C_{20}H_{40}O$	Phytol
5	15.84	2.45	262	$C_{18}H_{30}O$	9,12,15-Octadecatrienal
6	24.84	5.75	410	$C_{30}H_{50}$	Squalene
7	28.01	3.85	416	$C_{28}H_{48}O_2$	γ-tocopherol
8	31.45	10.45	370	$C_{25}H_{38}O_2$	9,12-Octadecodienoic acid
9	32.65	15.35	414	$C_{29}H_{50}O$	α-Sitosterol
10	33.56	6.15	426	$C_{30}H_{50}O$	α-Amyrin

CONCLUSION

The structure of *Ruellia brittoniana* powder is fine without any smell or odor. It has a unique color and microscopic characters with both simple and glandular trichomes, stomata, chlorophyll tissue, parenchymatous cells, calcium oxalate crystals, vascular elements, and epidermal cells. The fluorescent analysis of powder was unique and could be used as a marker to note the adulteration in market-available crude drug powder. The present study reveals that the plant *Ruellia brittoniana* is rich in phytochemical constituents especially alkaloids, glycosides, saponins, and carbohydrates, reducing sugar in all three solvents i.e., methanol, acetone, and petroleum ether. Except for petroleum ether; phenolics, flavonoids, terpenes, and tannins are found in the rest of the two extracts and proteins are absent in almost all the extracts. The availability of these groups of phyto-components indicates that the plant could prove an alternative remedy to cure asthma, fever, bronchitis, high blood pressure, eczema, and diabetes. The GC MS analysis helped to identify 10 phyto-compounds which are known to have antioxidant, anticancer, anti-inflammatory, anti-coronary, and anti-hepatic properties. Further, the pharmacological investigation is essential to confirm the medicinal potential of plant powder and its identified phyto -components.

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