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GC-MS analysis of phytochemical compounds in the crude methanolic extract of roots of *Murdannia lanuginosa* and *M. simplex* (Commelinaceae)

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The aim of the present study is to investigate the methanolic extract of the roots of *Murdannia lanuginosa* and *M. simplex* (Commelinaceae) for their phytochemical compounds using Gas Chromatography–Mass Spectrometry (GC-MS). The study reveals three compounds in *M. lanuginosa* and six compounds in *M. simplex* respectively. *M. lanuginosa* could be used for antimicrobial, anti-inflammatory and anti-proliferative activity due to the presence of secondary metabolites like 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl (Retention time 8.659) in the methanolic extract. While *M. simplex* extracts may be used in the development of fungicidal agent due to the presence of Salicylaldehyde, Azine (Retention time 22.608) as a major compound which is known for its fungicidal activity. A further study in the present taxa and family Commelinaceae is warranted to determine active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

Keywords: Azine, Commelinaceae, Fungicidal activity, GC-MS, Methanol extracts, *Murdannia*, Salicylaldehyde, Tuberous and thickened roots.

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Introduction

Humans depend on nature and natural resources to fulfill their basic needs. In addition, their interest in plants as a source of potential medicine/ therapeutic agents has continued over the centuries. Plants have formed the basis of traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies[1]. Herbal medicines have many advantages over synthetic counterparts. They are cost-effective and manifest better cultural acceptability and compatibility with the human body, presenting lesser side effects[2]. The emergence of new infectious diseases and rise in many metabolic disorders has prompted renewed interest in the discovery of potential drug molecules from medicinal plants[3, 4].

India is one of the 12 mega biodiversity centers with more than 45,000 plant species. Among these, about 1500 plants with medicinal uses are mentioned in ancient texts and around 800 plants have been used in traditional medicine. They are fundamental sources of several new chemical entities for development of drugs[3]. On the other side, more than 30,000 Indian species remain unexplored for their chemical constituents and pharmaceutical potential.

Genus *Murdannia* is one of the important groups of plants from the family Commelinaceae. The genus comprises of 55 species worldwide[4,5]. In India, there are 23 species enumerated by Karthikeyan *et.al.*[6] whereas the number of species was subsequently increased to 27 which include 7 endemic species[7,8]. The genus is well studied morphologically and has a high degree of endemism. Apart from this, few taxa were also evaluated for biochemical aspects. *Murdannia loriformis* (Hassk.) R.S. Rao & Kammathy has been evaluated for anti-inflammatory, analgesic, antipyretic, anticarcinogenic, antimutagenic, chemopreventive activities and gastroprotective potential[9-12]. *M. nudiflora* (L.) Brenan has been screened for phytochemical content and analgesic as well as anti-inflammatory activity[13]. Some species have been reported as indigenous herbal remedies, such as roots of *M. edulis* (Stokes) Faden in sexual disorder[14,15], while roots of *M. japonica* (Thunb.) Faden used in jaundice. Some of the *Murdannia* species are also noted for their ethnomedicinal use[16,17].

Chemical profiling of this genus is still unexplored and hence the present study endeavors to analyze methanolic extract of roots of *M. lanuginosa*

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(C.B. Clarke) G. Bruckn. (Fig. 1a) and *M. simplex* (Vahl) Brenan (Fig. 2a) by using GC-MS for their phytochemical compounds.

Materials and Methods

Collection and identification of plant materials

Fusiform roots of *M. lanuginosa* and thickened fibrous roots of *M. simplex* were collected from the Kas (Satara district, Maharashtra) and Morjai (Kolhapur district, Maharashtra). Both the species were identified and authenticated by using available literature[8]. Identified plant material accessions were deposited at Shivaji University Herbarium, Kolhapur (SUK) as MDN 25 [*M. lanuginosa*] and MDN 37 [*M. simplex*]. The thickened fibrous roots were labeled as [*M. lanuginosa* = ML; *M. simplex* = MS] and processed for shade drying and grinding. The collected plant materials were cleaned to remove mud

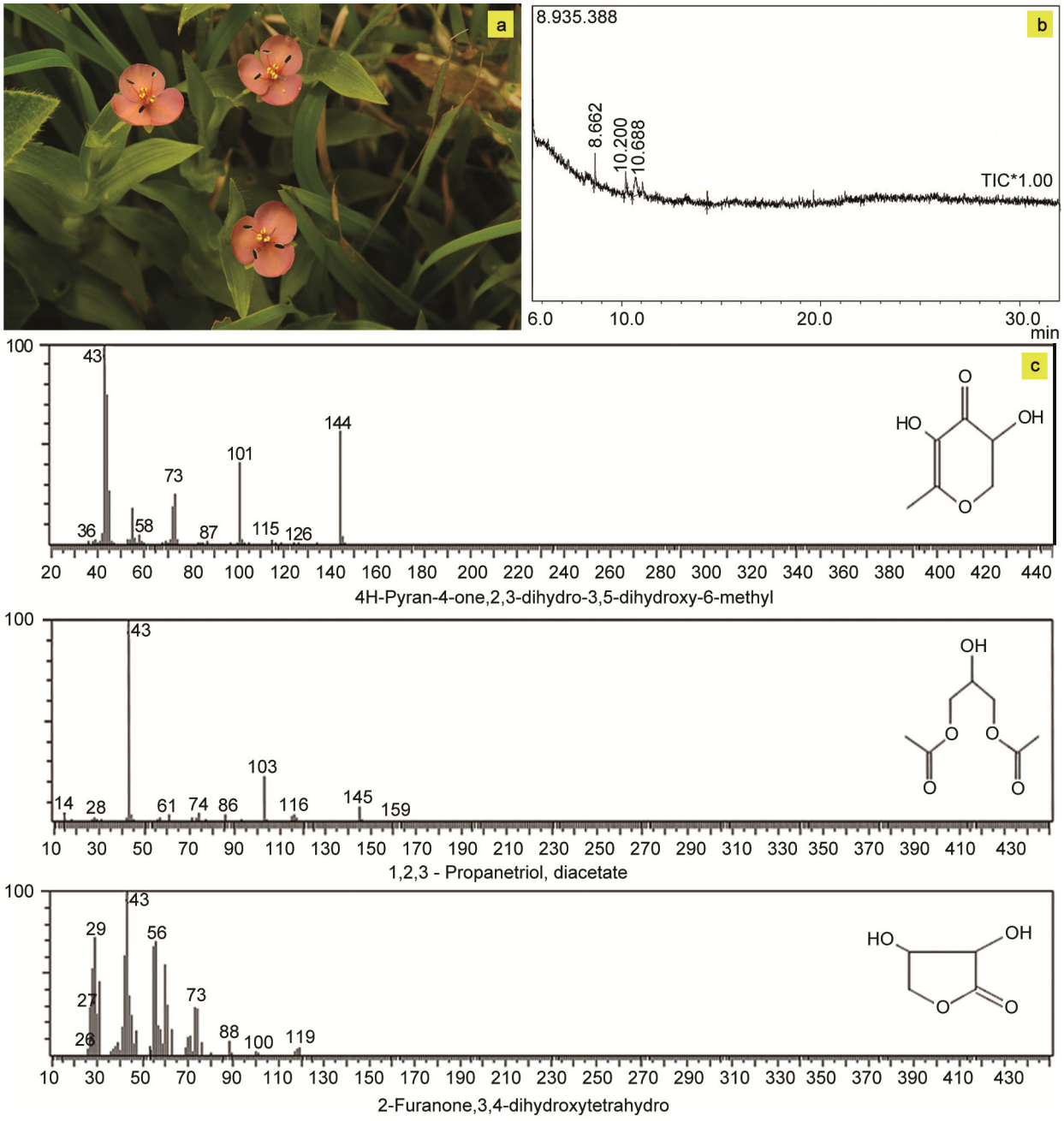

Fig. 1 — *Murdannia lanuginosa* a) habit, b) GC-MS chromatogram for methanolic extract, c) mass spectra of identified compound from methanolic extract

and other dirt. The cleaned plant materials were blotted and shade dried, and powdered using grinder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts

Extraction of powder have been made with methanol (analytical-grade obtained from SD Fine Chemicals, Mumbai, India) using soxhlet extractor. The extraction was carried out by using Soxhlet apparatus. The 20 g of plant material was placed in thimble which was kept in chamber of Soxhlet apparatus and extraction was carried out by 200 mL (1: 20 W/V) of solvent. The extraction was carried out repeatedly until solvent get colourless. The extract was concentrated by using rotary evaporator to dryness (Temp- 45 °C, RPM- 50, Vacuum Pressure- 15 mmHg). The dried extracts were stored at -20 °C until further use. The extracts which obtained were concentrated with a rotary evaporator and dry powder obtained. A stock solution of 1 mg/mL of ML and MS were prepared. Working solutions of both samples were prepared in the range 200 ng – 600 ng/mL by diluting the stock solution and applied for GC-MS analysis.

GC-MS analysis

GC-MS analysis was performed on a Shimadzu (Tokyo, Japan) Make QP-2010 with nonpolar 60 M RTX 5MS capillary column, full scan mode, injector mode-split, (split ratio 1:20), quadra pole mass selective detector (MSD), injection temperature 220 ºC, GC-MS interface temperature 230 ºC, the injection volume was 1 µL. Helium was employed as carrier gas, at a pressure of 60 KPa; flow rate was 1 mL/min. Mass spectra were detected at 70 eV. Temperature programming was set as follows: column temperature was started from 60 ºC (held for 2 minutes) and linearly increased by 5 ºC/min to 130 ºC (held for 2 minutes); after that it was increased by 4 ºC/min to 200 ºC (held for 2 minutes); further it was increased by 8 ºC/ min to 250 ºC (held for 10 minutes). Total GC running time was 30 minutes. The obtained data was analyzed by using GC-MS solution ver. 2.6 software. The detection employed in National Institute Standard and Technique 2005 library.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using Quadra pole detector with the database of National Institute Standard and Technique (NIST Version-Year 2005). The relative percentage amount of each compound was calculated by comparing its average peak area to the total areas. The spectrum of the unknown compound was compared with the spectrum of the known component stored in the NIST data library (version 2005). The name, molecular weight, molecular formula and structure of the components of the test material were determined.

Results

The genus *Murdannia* is most diversified and taxonomically well-studied plant group. Among which ML is endemic to the Western Ghats of India and occasionally grows on lateritic plateaus, rocky crevices and along the slope of the grasslands, and distinct from other *Murdannia* species by its fusiform tuberous roots and bi-seriate seeds. MS is a widespread species distributed throughout India, East Asia and Africa. It is found growing in elevated hills, grasslands and along the roadside ditches[8]. The species is distinct from other *Murdannia* species by its thickened fibrous roots and two-seeded locules.

In the present study, chemicals constituents are detected in respective extracts with their retention time (RT), molecular formula, molecular weight (MW), concentration (peak area %) and tabulated in Table 1 and 2. In the GC-MS analysis, cumulatively three bioactive phytochemical compounds from ML and six from MS have been identified. The mass spectra of identified compounds from ML and MS are represented in Fig. 1b and 2b.

In ML the major compounds analyzed are 2-Furanone, 3, 4-dihydroxytetrahydro (49.84 %; RT= 10.688) >

Table 1 — Phytochemical compounds identified in methanol extract of roots of *Murdannia lanuginose*

S. No	Retention time	Name of Compound	Molecular formula	Molecular weight	Composition (%)	Nature of compound	Activity
1	8.662	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	22.12	Flavonoid	Antimicrobial, anti-inflammatory, anti-proliferative[19]
2	10.200	1,2,3-Propanetriol,diacetate	$C_7H_{12}O_5$	176	15.77	Glycerol	Fragrance agents, cellular narcotic[20,21]
3	10.688	2-Furanone,3,4-dihydroxytetrahydro	$C_4H_6O_4$	118	49.84	-	-

Table 2 — Phytochemical compounds identified in methanol extract of roots of *Murdannia simplex*

S. No	Retention time	Name of Compound	Molecular formula	Molecular weight	Composition %	Nature of compound	Activity
1	8.659	4H-Pyran-4-one,2-3-dihydro-3,5-dihydroxy-6-mrthyl	$C_6H_8O_4$	144	1.28	Flavonoid	Antimicrobial, anti-inflammatory, anti-proliferative[19]
2	20.554	Bentranil	$C_{14}H_9NO_2$	233	3.02	Pyrimidine compounds	Herbicidal activity[24,25]
3	22.802	Salicylaldehyde,azine	$C_{14}H_{12}N_2O_2$	240	74.34	protamine	Fungicidal activity[22,23]
4	23.418	9,10-Anthracenedione,1-amino-2-methyl-Acetate Fast Orange R Acetoquinone Light Orange	-	237	4.75	-	-
5	24.976	Benzoic acid,2-benzoylhydrazide	$C_{14}H_{12}N_2O_2$	240	2.27	aromatic carboxylic acid	Antioxidant, antimicrobial activity[26]
6	31.038	Diphenylfurazan N-Oxide Furazan,diphenyl-,2-oxide 3,4-Diphenylfurazan N-oxide 3,4-Diphenylfurazan 2-oxide Diphenylfuroxan	$C_{14}H_{10}N_2O_2$	238	10.08	-	-

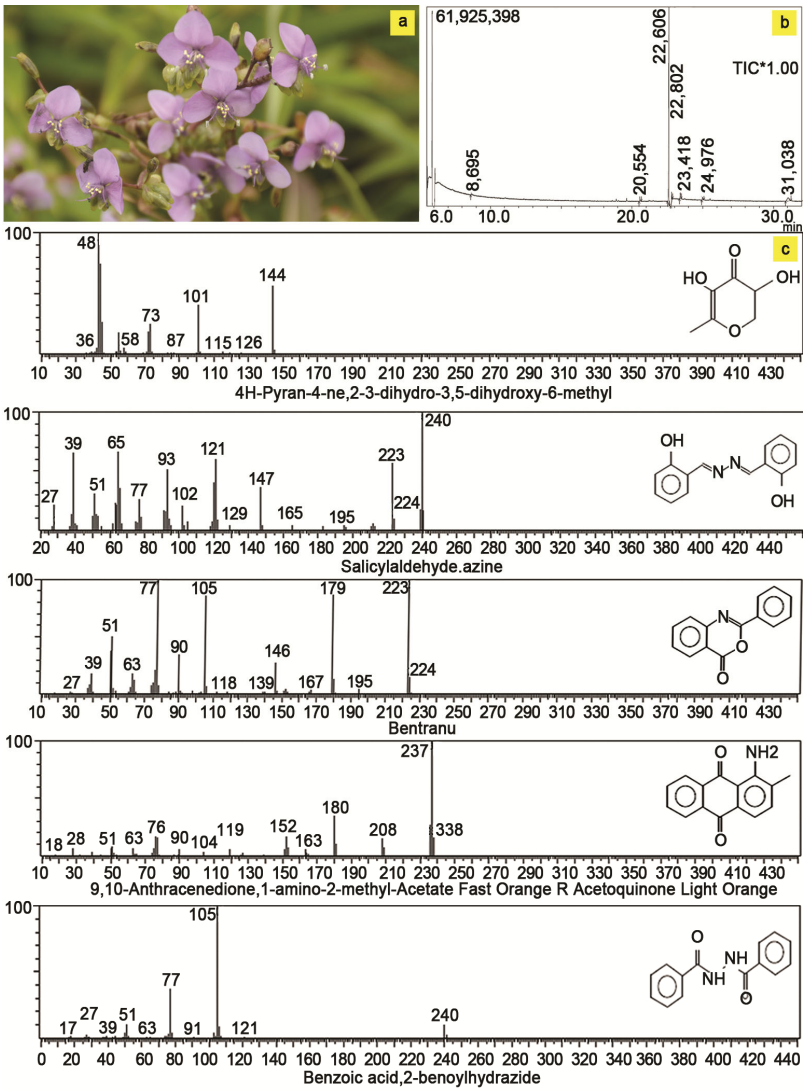

Fig. 2 — *Murdannia simplex* a) flowers, b) GC-MS chromatogram for methanolic extract c) mass spectra of identified compound from methanolic extract

4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl (22.12 %; RT= 8.662) > 1, 2, 3-Propanetriol, diacetate (15.77 %; RT= 10.200) (Fig. 1c). Among which 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl is a flavonoid in nature and studied for antimicrobial, anti-inflammatory, anti-proliferative properties[18]. 1, 2, 3-Propanetriol, diacetate is glycerol in nature and reported as fragrance agents which act as cellular narcotic[19].

In MS the major compounds are Salicylaldehyde, azine (74.34 %; RT= 22.802) > Diphenylfurazan N-Oxide Furazan, diphenyl-, 2-oxide 3,4-Diphenylfurazan N-oxide 3,4-Diphenylfurazan 2-oxide Diphenylfuroxan (10.08 %; RT= 31.038) > 9,10-Anthracenedione,1-amino-2-methyl-Acetate Fast Orange R Acetoquinone Light Orange (4.75 %; RT= 23.418) > Bentranil (3.02 %; RT= 20.554) > Benzoic acid,2-benzoylhydrazide (2.27 %; RT= 24.976) > 4H-Pyran-4-one,2-3-dihydro-3,5-dihydroxy-6-mrthyl (1.28 %; RT= 8.659) (Fig. 2c). On the basis of present analysis it is confirmed that MS methanolic extract is the foremost source of Salicylaldehyde, azine which is well known for fungicidal activity[21].

Conclusion

The occurrence of antimicrobial, anti-inflammatory and anti-proliferative activity of ML roots is due to the presence of secondary metabolites like 4H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl. It can be used further to synthesis, characterisation and biological evaluation, considering the interest in the development of novel compounds in the background of anti-inflammatory, antimicrobial activities[22]. Further, the presence of salicylaldehyde, azine in the thickened roots of MS which is one of the fungicidal active ingredients, mainly derived from the reaction with aldehyde or ketone and found to be effective in controlling *Cercospora* blights[21]. This can be further considered for isolation and quantification as an active fungicide. Hence the present work highlights the responsible phytochemicals present in the two *Murdannia* species and can be considered for future studies for the betterment of human health and welfare. The work also promises the phytochemical potentials in the family Commelinaceae, which is hitherto not materialized in the comprehensive approach.

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