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Qualitative Phytochemical Screening and GC-MS analysis of (*Eleusine coracana* (L.)

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ABSTRACT

The present investigation was carried out to evaluate the phytochemicals and GC-MS of seedlings of important cereal grain Eleusine coracana L., commonly called ragi. The dried germinated seeds powder was extracted separately with ethanol. The extracts were subjected to phytochemical analysis with different tests. Phytochemical analysis indicated the presence of significant phytochemicals viz, steroids, Glycosides, flavonoids, tannins, saponins, and volatile oils in the seedlings of all the genotypes of Ragi. Alkaloids were absent in all the genotypes studied. GC-MS analysis revealed five different bioactive compounds which are genotype-specific. The results revealed that the compound Trichloro ethylene was predominant in all the genotypes and the VR-847 genotype contained the least amount of bioactive components.

Keywords: *Phytochemicals, Ragi, GC-MS, ethanol, genotype, Alkaloids .*

INTRODUCTION

Finger millet (*Eleusine coracana* (L.) is avital millet cultivated in India and several African countries. It is very commonly known as ragi or Amanda in the Indian subcontinent. Finger millet is more nutritious than most cereal grains containing minerals, dietary fiber, and amino acids [1-3]. Ragi contains various phenolic compounds, including tannins that contribute to its excellent activity. Malt has a better food value than unmalted grain. Germination is a set of processes that leads to the birth of a plant from seed. Malting is the limited germination of cereal grains under controlled conditions [4-5]. Seed germination and post-germination seedling development are well-regulated processes involving high metabolic activity and ROS generation in the cell. ROS are known to have a dual role as both toxic products of aerobic metabolism and critical regulators of growth, development, and defence pathways [6-8]. Germination has a profound change in the nutritional quality of the cereal, and protein bioavailability is said to be increased. Since the consumption of seeds and sprouts is gaining popularity among people interested in improving and maintaining their health status, there is a necessity to study the impact of the traditional method of soaking and germination on the improvement of the nutritive value of cereal grains [9]. The seeds and sprouts are excellent examples of 'functional food', which lowers the risk of various diseases and promotes health benefits in addition to its nutritive value. Germinated ragi is are said to have better bioavailability of zinc and iron [10-12]. Because of this, the present investigation is aimed to evaluate the phytochemicals and bioactive compounds through GC-MS analysis from sprouts of ragi.

Materials and Methods

The dried seeds of ragi-*Eleusine coracana* GPV 67, ICMR-301, R-VL352, R-CF, and R-VR 847 are procured from Professor Jayashankar Telangana State Agricultural University, Hyderabad, India. The grain samples after collection were washed thoroughly first under tap water and then using distilled water to eliminate all the surface contaminants. From the collected samples about a few grams of seeds are used for cultivation with the help of trays which consists of good soil quality clay. The cultivars germinated within 3days and seedlings 15 days old with 6cm in length were taken for phytochemical study. The dried parts of the seedlings were pulverized by a mechanical grinder and passed through a 20µ sieve. 30g of each seedling sample is taken in separate beakers and 300ml of methanol is added to each beaker. These solvent extracts of seedlings are then agitated on a rotary shaker for about 48 hours. The contents of the beakers are mixed well and covered using aluminium foil. The beakers are then placed on a magnetic stirrer with a temperature between 25°-30°C and the extraction is continued for 72 hours. The extracts are filtered and stored for further use.

Phytochemical Analysis

The phytochemical analysis was carried out using the standard method [13-15] with minor changes.

Test for steroids: 2 ml of the extract was dissolved in 2 ml of chlororm and treated with concentrated sulphuric acid. The appearance of red color in the chloroform part confirms the presence of steroids.

Test for Glycosides

Keller-Killani test

In a test tube 2 ml solution of extract, 1ml glacial acetic acid, 5% $FeCl_3$ (3 drops), and concentrated H_2SO_4 were mixed and observed. The bluish-green color at the top and red color at the junction is due to the presence of cardiac glycosides.

Borntrager's test

In a test tube 2 ml of solution of extract and 2ml dilute H_2SO_4 were taken and heated. After filtration, chloroform was added, followed by ammonia. The pink color formation in the aqueous layer is due to Anthraquinonoid glycosides

Test for Volatile oils

Odor test: Characteristic odor of extract indicates the presence of volatile oil. Solubility test: Solubility in 90% alcohol indicates the presence of volatile oil.

Test for tannins

To 2ml of extract, add a few drops of ferric chloride solution. The occurrence of green-colored precipitate indicates the presence of tannins.

Test for saponins

To 2ml of extract, add about 2ml of distilled water and shake vigorously for a few seconds. The appearance of a stable foam indicates the presence of saponins.

Test for flavonoids

To 1 ml of extract in a test tube, add 1ml of 10% lead acetate. The formation of a yellow precipitate can be taken as a positive indication of the presence of flavanoids.

Test for Alkaloids

The plant extract is mixed, warmed, and filtered in 1% v/v HCl. To the filtrate, add Mayer's reagent. The presence of alkaloids is indicated by the formation of buff-colored precipitates.

GC-MS Analysis

The GC-MS analysis method was adopted according to Sparkman and David (2000). GC-MS analysis was carried out on a Shimadzu GCMSQ2010 ultra system, the injector temperature was 290°C. The samples were injected in the split mode with a split ratio of 1/23 and the injection volume was 1ul of the capillary column. The mass spectrophotometer settings were: 230°C for the ion source, 250°C for the interface, 4.5 minutes for the solvent delay, and 50–700 amu for the scan range. The multiplier voltage and electron ionization mode were tuned to 70 eV and 1859 V. For compound identification, the retention time, fragmentation pattern, and mass spectral data of unknown components in the

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extracts were compared to those in the Wiley and National Institute of Standards and Technology (NIST) libraries.

Table 1: Phytochemical analysis of Ragi varieties

S. No	Consituents	GPV-67	RC-F	VL-352	VR-847	ICMR-301
1	Steroids	+	+	+	+	+
2	Glycosides	+	+	+	+	+
3	Volatile oils	+	+	+	+	+
4	Saponins	+	+	+	+	+
5	Tannins	+	+	+	+	+
6	Flavonoids	+	+	+	+	+
7	Alkaloids	-	-	-	-	-

RESULT

The phytochemical analysis of the methanol extract of all five genotypes of the Ragi millet revealed the presence of steroids, glycosides, volatile oils, saponins, tannins, and flavonoids. However, alkaloids were found to be absent in all the genotypes of Ragi millets under study (Table 1). Similar results are reported by Bwai *et al*., (2014). The aromatic GC-MS chromatographic profiles of the millet chloroform extract showed various compounds in each genotype and were classified into different chemical classes. The compound Trichloro ethylene was found prominently as the major significant constituent

Table 2: Bioactive compounds of Ragi Millet Genotypes

S. No	Compound Name	R. Time	Mol Weight	Formala	CAS NO	GPV-67	RC-F	VL-352	VR-847	ICMR-301
1	Trichloro ethylene	1.494	131.38	C2HCL3	79-01-6	2.32	2.53	3.11	1.36	2.52
2	Cyclononasiloxane, octamethyl	27.920	667.14	C18H54O9SI9	556-71-8	5.29	1.51	--	--	--
3	Ethane 1,1,diethoxy	1.592	118.1742	C6H14O2	105-57-7	0.86	1.85	2.20	--	1.50
4	Aceticacid dichloro	1.330	128.942	C2H2Cl2O2	79-43-6	--	10.52	--	--	12.18
5	10Octadecenoicacid, Methyl ester	19.910	295.5	C19H36O2	13481-95-3	0.39	--	--	--	2.82

in all the genotypes GPV-67 (2.32), RC-F (2.53), VL-352 (3.11), VR-847 (1.36), and ICMR-301 (2.52) followed by Cyclononasiloxane, octamethyl GPV-67 (5.29) R-CF (1.51), Ethane 1, 1, diethoxy GPV-67(0.86), R-CF (1.51), VL-352(2.20), ICMR-301 (1.50), Acetic acid dichloro RC-F (10.52), ICMR-301(12.18), 10-Octadecenoic Acid, Methyl ester GPV-67(0.39), ICMR -301 (2.82), Acetic acid dichloro ICMR-301(12.18) contains the highest compound. 10-Octadecenoic Acid, Methyl ester (0.39) among all genotypes followed by the compound as the lowest of all genotypes. (0.39) (Table2).

DISCUSSION AND CONCLUSION

Millet is a highly nutritious cereal and the most widely consumed in the world. In addition, these crops also contain bioactive compounds, including carbohydrates, proteins, flavonoids, and other photochemical. The highest Iron concentration found in ragi millet suggests that the cultivation should be promoted as a food crop. The production of metabolites is a response to genetic and environmental changes. Plant terpenoids are used for their aromatic qualities. They play a role in traditional herbs and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Cardiac and anthraquinone glycosides are reported to have antibacterial and antifungal activity [16-18]. Tannins that are present in ethanol extract effect in vitro protein digestibility but it is unclear whether they are detrimental to animal nutrition [19-23]. Trichloroethylene was found in a remarkable amount in all genotypes followed by Cyclononasiloxane, octamethyl, Ethane 1, 1, diethoxy, Acetic acid dichloro, 10-Octadecenoicacid, and Methyl ester compound. And these tests can be used as a useful tool to analyze ragi seeds for their phenolic chemical lipid-rich components as a complete nutritional source. Finally, further research has to focus on a comprehensive profile of all the secondary metabolites of this important cereal crop. .

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Consent and Ethical Approval

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors

Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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