Evaluation of Phenolic Content and Antioxidant Capacity of *Eleusine coracana* (L.)

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Abstract: The aim of this study is to evaluate the content of phenolics: total seven phenolic acids and one flavonoid were separated from the species of *Eleusine coracana* (L.). The separated phenolics were characterized by various spectral techniques. The antioxidant capacity of all phenolics was determined by rancimat study of sunflower oil using control and standard drugs like Glipizide and Metformin. The stability period of sunflower oil was found to increase from 0.89 hr to 1.04 hr in presence of eight extracted ingredients of *Eleusine coracana* (L.), which was compared with result of neat sunflower oil.

Keywords : Antioxidant capacity, sunflower oil, phenolics, *Eleusine coracana* (L.), semi-Prep-HPLC analysis.

Introduction

Eleusine coracana (L.) is native to East African highland, India and China and is considered as one of the richest sources of phenolics¹ and it contains flavonoides, which have high biological activity². Many synthetic antioxidants are available but these compounds must be used under strict regulation due to their potential hazards^{3, 4}. Therefore, new interest has been developed as purifying and characterizing safe antioxidants from natural sources. The predominant flavonoides and phenolic acids, which are almost exclusively present in *Eleusine coracana* (L.), are linked with glycosides⁵.

There have been many attempts made to determine the contents and physiological activity of phenolic compounds present in *Eleusine coracana* (L.) due to the apparent relationship of phenolics in minor millet with prevention of chronic diseases. Phenolics are natural secondary metabolites and are widely used as potential components for hypertension, diabetes, heart diseases, osteoporosis, and some forms of cancer, avian flu, Endometriosis, chronic fatigue syndrome, tetanus, different types of cancers, lyme disease, chronic ear infection and even obesity are considered chronic diseases⁶. There are many factors like

surrounding atmosphere, environmental conditions and soil conditions, which affect the phenolics and their antioxidant capacity of *Eleusine coracana* (L.).

Minor millets are rich in phytochemicals, including phytic-acid and phytate, which are believed to lower cholesterol and cancer risk⁷. *Eleusine coracana* (L.) is well known minor millet for its anti-nutrient constituents such as trypsin inhibitors, phytates, phenols and tannins⁸. Unfortunately, very less attention has been given to this millet for exploring its medicinal properties and therefore, the present study; as part of the continuing investigation on the antioxidant constituents of minor millet, deals with the separation and identification of the minor anti-oxidative components found in *Eleusine coracana* (L.)^{9,10}.

Even though, some components occur only in minor concentrations in crude extracts, they could be strong antioxidants, which make them interesting for further purification and identification. Minor anti-oxidative components from *Eleusine coracana* (L.) were detected and separated using semi-prep-HPLC-PDA technique followed by GC-MS for their characterization¹¹. The structure elucidation of the eight components and their overall antioxidant activity were reported herein. Thus, the general purpose of this work is to extend our knowledge on the chemical compositions of *Eleusine coracana* (L.). This would be a first step to assess the precision of this agricultural product as a source of natural antioxidants, potentially useful for the food industry to prepare functional foods or as natural antioxidant additives from *Eleusine coracana* (L.)¹².

Experimental

Materials and Chemicals

Eleusine coracana (L.) was collected from Millet Research Centre, Waghai, Gujarat-India with proper authentication under the supervision of Botanist. The collected samples were preserved in dark and dry place at ambient temperature with passive ventilation prior to extraction.

Silica gel 60 (Merck) with particle size 40– 65 μm was used for column chromatography, while silica gel 60 F₂₅₄ pre-coated aluminum sheets (0.25 mm, Merck) were employed for TLC. HPLC analysis and separation were conducted on a quaternary gradient HPLC system containing W600e multi solvent delivery system from Waters, a degasser with Helium and 2996-photodiode array detector with EMPOWER 2 software. The system was equipped with a normal phased semi-preparative column Xterra MSC18 (7.8 $\times$ 100 mm; 5 μM) and flow rate was kept one $\text{mL}\cdot\text{min}^{-1}$ for all the experiments performed. HPLC solvent A was methanol (gradient grade) and solvent B was 10 mM ammonium acetate with pH adjusted to 4.14 using glacial acetic acid. The solvents used for extraction of active ingredients from *Eleusine coracana* (L.) (1% acidified methanol) and chromatographic separation were glass-distilled prior to use.

Extraction of phenolics from Eleusine coracana (L.)

The phenolics of *Eleusine coracana* (L.) were extracted successively with the help of 1 % acidified methanol solvent of HPLC grade using Accelerated Solvent Extractor (ASE) technique with the aid of ASE-300 multi channel system from Dionex, Germany. Ottawa sand was used to remove interference from moisture. The extraction conditions were optimized based on different percentage recovery of extracts in the different experimental conditions. The solvent was evaporated under reduced pressure to give the maximum percentage yield from *Eleusine coracana* (L.). An aliquot of 1%-acidified methanol was chromatographed by open column chromatography on silica gel, using chloroform: methanol mixtures of increasing polarity. Fractions of 500 mL in 10 mL sub-fractions in tagged vials were collected, monitored by thin-layer chromatography (TLC), to identify

eight major constituents, which were subsequently given the numbers as C-1, C-2, C-3, C-4, C-5, C-6, C-7 and C-8.

An aliquot of crude fraction was chromatographed by semi-preparative HPLC. A linear gradient from 5% - 50% and back to 5% of solvent B in 90 min was used to yield pure fractions from crude extracts of *Eleusine coracana* (L.).

Standard and sample solution preparation

Standard drugs were purchased from local market, dissolved and diluted up to one $\mu\text{g.mL}^{-1}$ in methanol and these solutions were centrifuge, filtered and stored for further use. The separated phenolics were dried after extraction and dissolved in methanol for obtaining 1 mg.mL^{-1} stock solutions of phenolics.

Purification of antioxidant

Semi prep-HPLC-PDA technique is extremely useful for the analysis of natural products containing phenolics. The chromatogram at multiple wavelengths was retrieved from the data files after analysis¹³. The HPLC retention time and the UV-VIS spectrum for any component (LC peak) are characteristic of certain compounds. The data are rapidly previewed for unique absorption regions correlating to specific compounds or functional groups^{13,14}. Quaternary gradient semi-prep HPLC system with PDA detector 600e multi solvent delivery system from WATERS (EMPOWER 2) was used along with column Xterra MSC18 (7.8 $\times$ 100 MM, 5 μm). PDA detector (2996 Photodiode Array Detector) was used and the specific wavelength used for detection of phenolics is 236 nm.

Results and Discussion

Optimization of solvent and time was performed with six different solvents having different polarities to achieve maximum percentage yield of crude extract of *Eleusine coracana* (L.) for phenolic content and their results are summarized in Table 1. It was found that 1% acidified methanol is more suitable solvent for extracting the phenolics from *Eleusine coracana* (L.).

Table 1. Optimization data for maximum Extraction yield in ASE.

Time (Min.)	Recovered mass (gm)				
	n-H	R.S.	n-H & R.S.	n-H & methanol	Methanol(1% HCl)
5	0.0518	0.1767	0.1182	0.2693	0.2551
10	0.0547	0.1964	0.1478	0.2958	0.3449
15	0.1295	0.2872	0.1548	0.3383	0.3846
20	0.1584	0.3206	0.2638	0.3546	0.4928
25	0.2393	0.3534	0.3165	0.3872	0.5423
30	0.2672	0.4101	0.3569	0.4271	0.5962

Reproducibility of Standards by semi-prep-LC- PDA techniques

Reproducibility of phenolics is verified by semi-prep LC-PDA. The chromatograph of mix standards under specified experimental conditions is depicted in fig.1¹⁵, which is of suggestive that separation of standards were optimized by this method.

Figure 1. HPLC chromatogram of mixture of eight standards in gradient elution with one mg.mL^{-1} concentration.

Figure 2. HPLC chromatogram of *Eleusine coracana* extract in gradient elution with one mg.mL^{-1} concentration.

To authenticate the data total eight injections of 5000 μL of eight standards were injected¹⁶. The average retention time (t_R) with their standard deviation and % RSD clearly suggested that all the eight components were separated under the similar experimental conditions with fine distinct peaks as described in the Table 2. Therefore, same practice was carried out for actual sample analysis as described herein^{17,18}. The actual sample injection under similar conditions leads to separation of phenolics from crude extract of *Eleusine coracana* (L.) and a chromatogram is shown in the fig.2. Out of twelve separated components, eight were identified based on our results of standards and summarized data are depicted in the Table 3¹⁹⁻²¹.

Table 2. Retention time, Deviation, Standard deviation and percentage RSD for Individual standards in the mixture of eight components.

No. of Injection	Retention Time (t_R) for individual standards in standard mixture						
	Gallic acid	Caffeic acid	Vanillic acid	Cinnamic acid	Ferulic acid	PHBA	Quercetin
	C ₁	C ₂	C ₃	C ₄	C ₅	C ₇	C ₁₃
1	4.696	40.289	31.924	54.425	48.710	19.076	58.739
2	4.696	40.292	31.920	54.420	48.703	19.070	58.733
3	4.697	40.291	31.925	54.424	48.696	19.073	58.735
4	4.692	40.289	31.923	54.425	48.700	19.076	58.739
5	4.696	40.289	31.922	54.418	48.700	19.069	58.743
6	4.699	40.295	31.932	54.434	48.710	19.083	58.745
7	4.694	40.280	31.926	54.428	48.698	19.078	58.737
8	4.696	40.292	31.917	54.425	48.694	19.072	58.730
Average	4.696	40.290	31.924	54.425	48.701	19.075	58.738
Deviation	2.95×10^{-5}	1.36×10^{-4}	1.38×10^{-4}	1.65×10^{-4}	2.50×10^{-4}	1.48×10^{-4}	1.74×10^{-4}
Standard Deviation	0.00314	0.00673	0.00678	0.00741	0.00913	0.00702	0.00761
RSD (%)	6.678	1.670	2.124	1.362	1.874	3.681	1.296
Data for peak area							
Average	483390	4818114	224703	3372339	2402044	3846647	379308
Deviation	1.48×10^9	9.75×10^{10}	2.50×10^7	1.15×10^{10}	1.87×10^{10}	1.27×10^{11}	1.03×10^9
RSD (%)	3.01	2.45	0.84	1.20	2.15	3.50	3.20

The statistical analysis is done by using STAST software programme available in the Department of Statistics, Bhavnagar University.

Rancidity of Sunflower oil

Oils can be particularly susceptible to rancidity because their chemistry, which makes them susceptible to oxygen damage. During the process of oxidative rancidity, oxygen molecules interact with the structure of the oil and damage its natural structure in a way that can change its odour, its taste, and its safety for consumption.

Table 3. HPLC Chromatographic data of *Eleusine coracana* (L.) extract.

Peak No	(t _R) in (min)	Area	% Area	Height	Wave length (λ)	Identified compound
1	5.888	16829	1.35	392	261.9	Gallic acid
2	19.672	271654	21.77	3714	251	PHBA
3	24.612	240703	19.29	2318	230.9	Unknown
4	31.95	27671	2.22	269	255.7	Vanillic acid
5	40.986	305475	24.48	3285	316.2	Caffeic acid
6	42.849	40239	3.23	1001	229.7	Unknown
7	47.682	24069	1.93	973	213.3	Unknown
8	48.916	89160	7.15	3285	216.8	Ferulic acid
9	50.156	1263	0.1	108	285.3	Unknown
10	52.036	28922	2.32	648	238.0	Unknown
11	54.578	177264	14.21	5327	273	Cinnamic acid
12	58.793	24378	1.95	676	368.6	Quercetin

Rancidification is the decomposition of fats, oils and other lipids by hydrolysis or oxidation, or both. Oxidation primarily occurs with unsaturated fats by a free radical-mediated process. These chemical processes can generate highly reactive molecules in rancid foods and oils, which are responsible for producing unpleasant and noxious odours and flavors. These chemical processes may also destroy nutrients in food. Antioxidants are often added to fat-containing foods in order to retard the development of rancidity due to oxidation. Fresh 5.0 gm samples of sunflower oil were taken into vessel of rancimat and were subjected to five different temperature programmers because increased in the temperature increased the rate of oxidative rancidity in the oil. It was found that at 140°C sunflower oil became rancid after 1.11hr, while oxygen damage is found rapid in the presence methanol and sunflower oil became rancid within 0.89 hour under similar experimental conditions. Two standard drugs Glipizide and Metformin²²⁻²⁵ were also used under similar conditions for their induction time and sustainability. It was found that presence of standard drugs increased the stability time from 0.89 hr to 1.04 hr suggests that standard drugs have good impact on rancidity of sunflower oil because both drugs able reduce the oxygen damage in the sunflower oil^{10,18}. Similarly, eight components (C1-C8) were also used to improve the stability time for sustaining the oxidative rancidity. The stability time for sunflower oil was increased from 0.89 hr to 1.04 hr, which indicates that natural antioxidants extracted from *Eleusine coracana* (L.) have great impact in the

reduction of induction time and increased the stability of oil considerably by decreasing the rate of oxygen damage or rancidity. The rate of induction time and stability period from crude extract of *Eleusine coracana* (L.) and standard drugs is depicted in fig. 3. The data for oxidative rancidity of sunflower oil with MeOH, standard drugs and crude extracts is summarized in the Table 4.

Figure 3. (a) Rate of rancidity of sunflower oil in the presence of methanol; (b) Rate of rancidity of sunflower oil in the presence of methanol and standard drug Glipizide; (c) Rate of rancidity of sunflower oil in the presence of methanol and extracted ingredients of *Eleusine coracana* (L.).

Table 4. Summarized data of Rancidity of sunflower oil in the presence of media, standard drugs and *Eleusine coracana* (L.) extract.

ID 2	ID 1	Temp. (°C)	Rancid time (Hrs.)
Sunflower oil	-----		1.11
Blank media	Sunflower oil + MeOH		0.89
Standard drug 1	Blank media + Glipizide	140 °C	1.03
Standard drug 2	Blank media + Metformin		1.03
Sample	Blank media + <i>Eleusine coracana</i> (L.)		1.04

Conclusion

Neat sunflower oil became rancid in 1.11 hr at 140°C, whereas Sunflower oil became rancid in 0.89 hr in the presence of methanol, indicates rapid oxygen exchange between both molecules. Under similar conditions, the stability time was found increased in the presence of two standard drugs and stability of an oil was increased from 0.89 hr to 1.04 hr. Similarly, extract of *Eleusine coracana* showed good antioxidant activity when compared with standard drugs like those that Glipizide and Metformin and stability time was found increased up to 1.04 hr. It is also evident from the study that natural antioxidants are very much useful as an additive to sustain the food gradients for longer period and therefore, crude extract of *Eleusine coracana* should be explored by the food industries as source of natural antioxidants.

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