Phytochemical and antioxidant characterization of ethanolic extract of Zea mays

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ABSTRACT

The antioxidant activity and phytoconstituents of Zea mays cob extract were investigated in this study. GC-MS analysis was employed to examine the extract and total 47 phytochemical components were identified. The retention times and peak areas of these compounds were compared to literature data for their identification. Among the identified constituents, Campesterol (1.13%), Guanosine (2.19%), and Isochiapin B (0.43%) were found to be the most prevalent chemical ingredients.

To evaluate the antioxidant potential of the ethanolic extract, the DPPH technique was employed. At a concentration of 50 µl, the extract displayed a maximal antioxidant activity of 61.0323%, with an IC50 value of 38.8881 µl, indicating its capability to scavenge DPPH radicals effectively.

This research represents the initial observations, demonstrating that Zea mays cob extract possesses high levels of phytochemicals, contributing to its potent antioxidant properties. Further investigations may provide valuable insights into the applications and mechanisms of this extract in various fields.

KEY WORDS: Zea mays, GC-MS, Antioxidant activity, DPPH assay, free radicals

INTRODUCTION

Maize (Zea mays L.) is a major annual grain crop in the world that belongs to the Poaceae family [1]. Maize (Zea mays L.) is the most commonly produced and productive cereal crop in the world, coming in first place among the three primary food crops (maize, wheat, and rice). In China, maize is a major food, feed, and cash crop, and it contributes significantly to the country's agricultural output. Maize is also important for blood pressure control, liver protection, and fatigue recovery. The internal metabolites and metabolic processes of maize are involved in all of these effects [2].

Corn seeds are used as a source of nutrition by humans, while the stem and leaves are used as cattle fodder all over the world. Corn silk and cobs are frequently thrown away as garbage. A variety of bioactive phytochemical substances with antioxidant properties can be found in all sections of the maize plant. It produces over 780 million metric tonnes annually, with the United States, China, Brazil, and India being the largest producers. It's a 2–20-foot-tall annual herbaceous plant. Z. diploperennis, Z. luxurians, Z. nicaraguensis, Z. perennis, and Z. mays are the five species that make up the Zea genus. The only cultivated grass is Zea mays, with the rest being wild grasses. In Asian countries such as China, Korea, Taiwan, Vietnam, Laos, Myanmar, Thailand, India, and Pakistan, corn seeds are used as a food [3]. Alkaloids, flavonoids, saponins, maizenic acid, vitamins B1, K, and potassium, phosphorus, and zinc are all found in the plant [4]. Zea mays biological properties include blood pressure control, cholesterol control, and cardiovascular disease prevention, as well as antioxidant, antibacterial, anticancer, atherosclerosis, hyperlipidemia, diabetes, and obesity prevention [5]. The ash of the cob is used for the treatment of cough [6].

The DPPH (α, α-diphenyl-β-picrylhydrazyl) free radical scavenging method is the first method for determining the antioxidant capacity of a chemical, an extract, or other biological sources. The most basic method involves mixing the potential chemical or extract with DPPH solution and measuring the absorbance after a set amount of time. However, as instrumental techniques have advanced and become more sophisticated, the process has undergone different alterations to fit the needs, even though the core concept has remained consistent [7]. For decades, Gas chromatography–mass spectrometry (GC-MS) has been employed to analyse biological samples. The US National Institute of Standards and Technology and other agencies use this technique to develop definitive methods for qualifying standard reference materials and assigning accurate concentrations to reference materials of a variety of clinically relevant analytes, including cholesterol,

glucose, steroid hormones, creatinine, and urea nitrogen [8].

MATERIAL AND METHODS:

Procurement and verification of plant material

Zea mays cobs were purchased from a local market in Lucknow in December 2021. Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Integral University, Lucknow, India 226026 authenticated the cob sample.

Chemicals

The analytical grade chemicals were used in the research study which includes solvents for extraction and purification.

Extraction of plant material

The dried cobs of Zea mays were ground to coarse powder (100 g); soaked in ethanol at room temperature with occasional shaking and filtered. The filtrate was dried to a thick semi-solid pasty substance of dark brown colour using a rotary evaporator under reduced pressure. GCMS and DPPH analysis were performed on the extract [9].

GC-MS analysis of phytoconstituents

Gas chromatography-mass spectroscopy was used to investigate an ethanolic extract of Zea mays cob (GC-MS). The Shimadzu QP-2010 Ultra GC-MS instrument used to have a capillary standard and a non-polar column of 60 M TRX 5-MS (Dimension: 30 m, ID: 0.25 mm, film: 0.25 mm). The vehicle gas was helium, and the mobile phase flow rate was held constant at 1.21 ml min-1. The temperature of the device's oven was originally raised from 100 to 260 ° C. at a rate of 10 ° C. per minute, and the per injection volume was set at 2 µL. 70 eV was used to test the electron ionisation energy system. The sample EEZM was given a 60-minute flow time. To dissolve the sample EEZM, hexane was used, that ran at a range of 10–850 m/z. With the use of the Wiley spectral library search database, the observations were noted and subsequently analyzed-equated. The mass spectra were obtained over duration of 30–35 minutes. The comparative proportion of each metabolite was calculated by equating its average peak area to the total areas. The class identification of detected distinct components was established by comparing m/z ratio with (Sigma-Aldrich) authenticated sample together with mass spectra data in the NIST Mass Spectral Library Ver. 2.0 d (2005) and literature [10].

DPPH radical scavenging assay

For this activity 2mM DPPH solution in methanol was used, and sample extract (EEZM) were prepared for 1ml volume in methanol, where the sample was added as 10, 20, 30, 40 and 50µl and volume made up to 1ml. So, for the sample tubes containing 1ml of sample extract was loaded with 2ml of 1mM DPPH solution and the content was vortexed properly. The tubes were then incubated at room temperature in dark for 30minutes and then optical density of each sample tube was measured against methanol as blank at 517nm using UV-Visible double beam spectrophotometer. A control tube that is the freshly prepared 1mM DPPH solution was made and its optical density was measured immediately at 517nm [11,12].

To determine the % scavenging activity the below given formula was used;

$$\% \ Scavenging = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

RESULTS

Phytochemical analysis

The extraction of plant material and subsequent analysis are crucial steps in the development of herbal composition because they assure consistency and quality control. The current study used Gas chromatography-Mass spectroscopy (GC-MS) to detect biologically active chemicals present in an ethanol extract of Zea mays cob. The result concludes presence of various polar and non-polar bioactive compounds. In Zea mays cobs, in total 47 kinds of phytoconstituents were scrutinized, **fig. 1** represented the GC-MS chromatograms achieved for SC. The active principle, area of the peak, concentration, and retention time are presented in **Table 1**. The existing compounds idenitified in n-hexane extract were 3-Deoxy-d-mannoic lactone(9.43%), Stigmast-5-en-3-ol, (3beta,24S)-(9.13%), Dodecane, 1-chloro-(8.43%), Diethyl Phthalate(7.00%), Tetradecane, 1-chloro- (6.95%), n-Hexadecanoic acid(6.58%), Stigmasta-5,22-dien-3-ol (6.45%), 1-Dodecanol (5.57%), 5-Hydroxymethylfurfural(4.34%), 4H-Pyran-4-one, 3-hydroxy-2-methyl- (4.18%), Guanosine(2.19%), 1,2,3-Propanetriol, 1-acetate(2.99%), Heptanoic acid, 6-oxo-(2.34%), 1,2,3-Propanetriol(1.37%), 1,2,3,4-Butanetetrol, [S-(R*,R*)]-(1.86%), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (1.70%), 2-Methoxy-4-vinylphenol (1.45%), 9-Octadecenoic acid (Z)-, methyl ester (1.15%), 1,2-Cyclopentanedione(1.11%), Campesterol (1.13%), Hexadecanoic acid, methyl ester (1.02%), 10-Undecenyl hexofuranoside (1.10%), Benzofuran, 2,3-dihydro-(0.92%), 9,12-Octadecadienoic acid (Z,Z)-(0.86%) Octadecyltriethoxysilane(0.79%), Dodecyl 4-methylbenzenesulfonate (0.74%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (0.61%), 1,40-tetracontanediol(0.61%), Docosanoic acid, docosyl ester (0.60%), 1-tridecanol(0.57%), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydr(0.55%), 2-Acetyl-2-hydroxy-.gamma.-butyrolactone (0.50%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one(0.56%), α-D-Glucopyranoside, methyl (0.47%), Isochiapin B (0.43%), Z,Z-8,10-Hexadecadien-1-ol(0.41%), 3-Methyldihydro-2,5-furandione (0.41%), 5,9,13,17-Tetramethyl 4,8,12,16-octadecatetraenoic acid(0.38%), 2-Dodecyl-5-methylpyrrolidine(0.37%), Butanedioic

acid, 2-hydroxy-2-methyl-, (S)-(0.36%), Z,Z-3,15-Octadecadien-1-ol acetate(0.33%), Tricosanoic acid, methyl ester(0.34%), n-Pentadecanol(0.32%), 7-Hexadecenal, (Z)- (0.28%), Doosane(0.26%), 3(2H)-Furanone, 4-hydroxy-5-methyl-(0.23%).

Figure 1: GC-MS chromatogram of ethanolic extract Zea mays cob (EEZM)

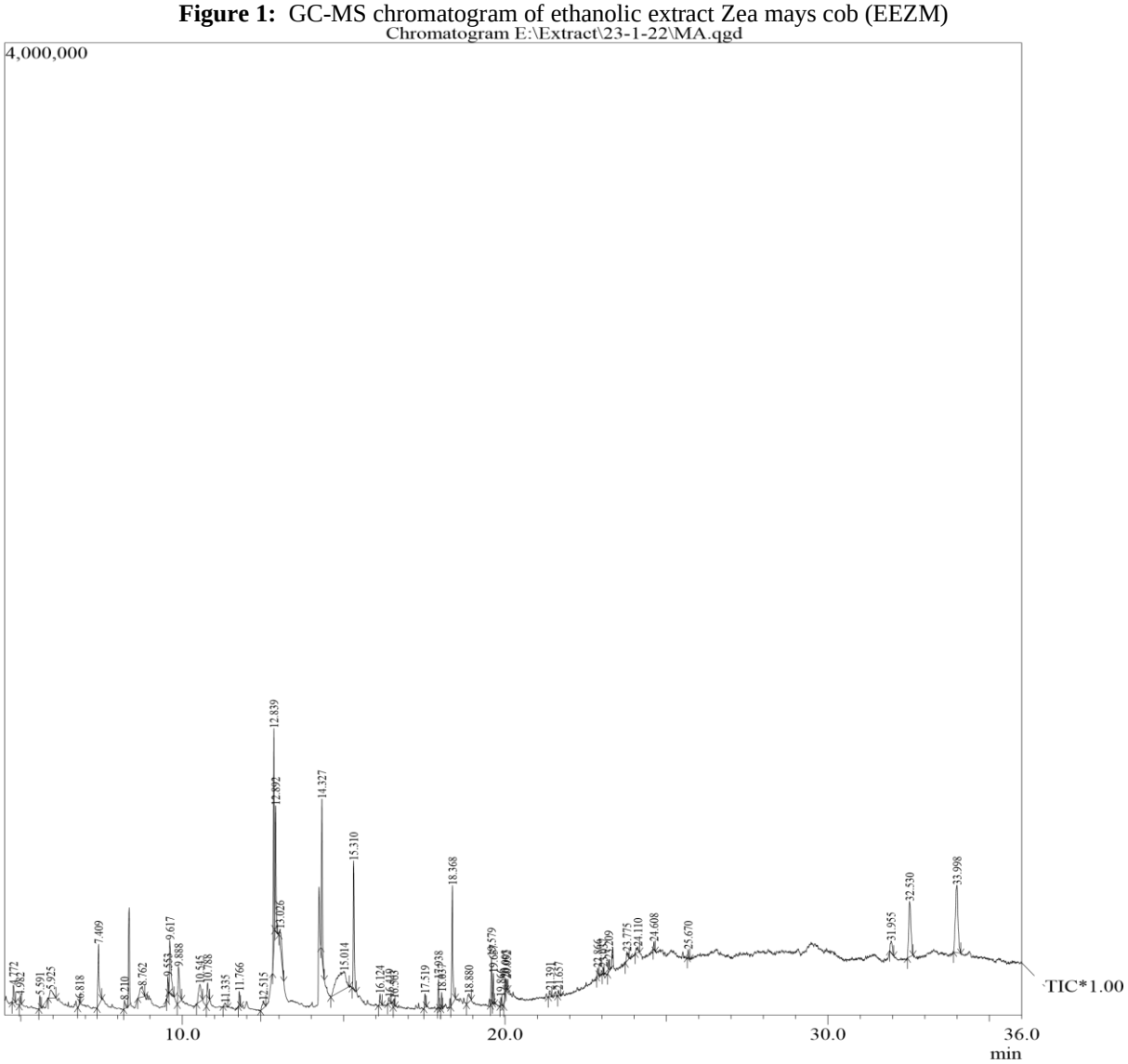


Table 1: Compounds present in ethanol extract of Zea mays cob (EEZM) by using GS-MS analysis.

Peak	R.Time	Area	Area%	Name
1	4.772	188606	1.11	1,2-Cyclopentanedione
2	4.982	70115	0.41	3-Methyldihydro-2,5-furandione
3	5.591	95379	0.56	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4	5.925	233078	1.37	1,2,3-Propanetriol
5	6.818	38910	0.23	3(2H)-Furanone, 4-hydroxy-5-methyl-
6	7.409	710643	4.18	4H-Pyran-4-one, 3-hydroxy-2-methyl-
7	8.210	85841	0.50	2-Acetyl-2-hydroxy-.gamma.-butyrolactone
8	8.762	317079	1.86	1,2,3,4-Butanetetrol, [S-(R*,R*)]-
9	9.553	156374	0.92	Benzofuran, 2,3-dihydro-
10	9.617	737587	4.34	5-Hydroxymethylfurfural
11	9.888	508759	2.99	1,2,3-Propanetriol, 1-acetate

12	10.545	397650	2.34	Heptanoic acid, 6-oxo-
13	10.788	246855	1.45	2-Methoxy-4-vinylphenol
14	11.335	61460	0.36	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-
15	11.766	96509	0.57	1-Tridecanol
16	12.515	134829	0.79	Octadecyltriethoxysilane
17	12.839	1433821	8.43	Dodecane, 1-chloro-
18	12.892	947458	5.57	1-Dodecanol
19	13.026	372877	2.19	Guanosine
20	14.327	1190110	7.00	Diethyl Phthalate
21	15.014	1603550	9.43	3-Deoxy-d-mannoic lactone
22	15.310	1181414	6.95	Tetradecane, 1-chloro-
23	16.124	108423	0.64	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol
24	16.419	79370	0.47	α-D-Glucopyranoside, methyl
25	16.563	54989	0.32	n-Pentadecanol
26	17.519	101985	0.60	Docosanoic acid, docosyl ester
27	17.938	172880	1.02	Hexadecanoic acid, methyl ester
28	18.037	94068	0.55	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydr
29	18.368	1120095	6.58	n-Hexadecanoic acid
30	18.880	186722	1.10	10-Undecenyl hexofuranoside
31	19.579	289632	1.70	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
32	19.637	195818	1.15	9-Octadecenoic acid (Z)-, methyl ester
33	19.866	57450	0.34	Tricosanoic acid, methyl ester
34	20.003	146671	0.86	9,12-Octadecadienoic acid (Z,Z)-
35	20.052	69545	0.41	Z,Z-8,10-Hexadecadien-1-ol
36	21.391	73223	0.43	Isochiapin B
37	21.657	55568	0.33	Z,Z-3,15-Octadecadien-1-ol acetate
38	22.866	63395	0.37	2-Dodecyl-5-methylpyrrolidine
39	23.037	44992	0.26	DOCOSANE
40	23.209	103866	0.61	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
41	23.775	125147	0.74	Dodecyl 4-methylbenzenesulfonate
42	24.110	103830	0.61	1,40-tetracontanediol
43	24.608	47444	0.28	7-Hexadecenal, (Z)-
44	25.670	64547	0.38	5,9,13,17-Tetramethyl 4,8,12,16-octadecatetraenoic acid
45	31.955	191949	1.13	Campesterol
46	32.530	1096307	6.45	Stigmasta-5,22-dien-3-ol
47	33.998	1552999	9.13	Stigmast-5-en-3-ol, (3beta,24S)-
		17009819	100.00	

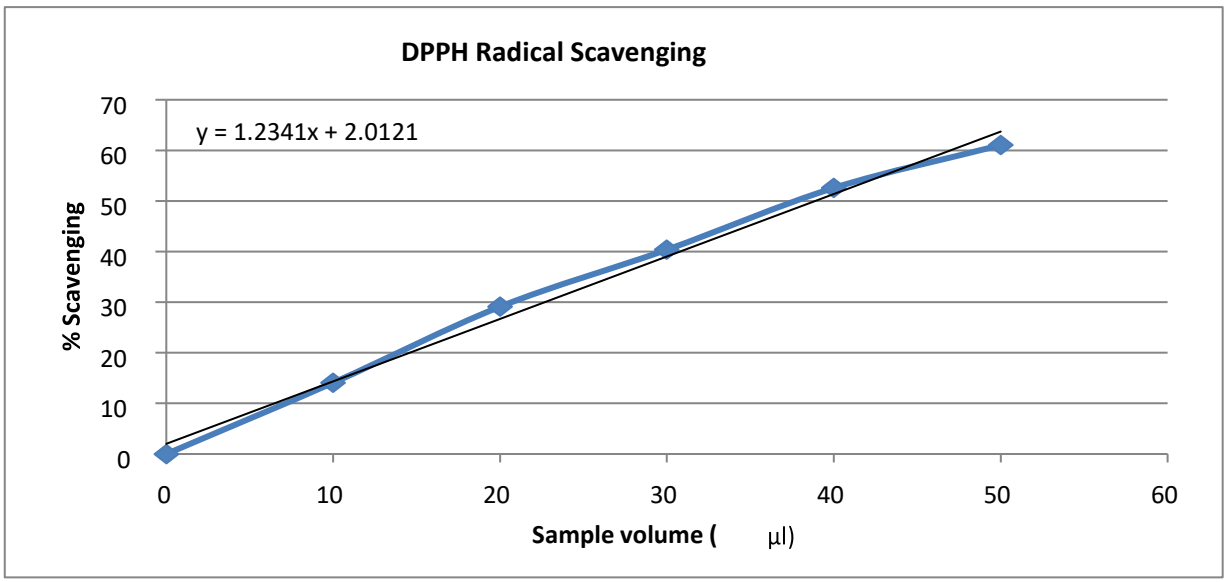
Antioxidant activity

A graph (**fig. 2**) was plotted against sample volume and % scavenging; the equation generated on the graph was used for calculation of IC50 value. **Table 2** summarizes the DPPH radical scavenging activity of ethanolic extracts Zea mays cob (EEZM) showed 14.08450704%, 29.10798122% , 40.37558685% , 52.58215962% , 61.03286385% of scavenging activity at concentrations 10, 20, 30, 40, 50 µl respectively. Its maximum radical scavenging was found to be 61.032% at a concentration of 50 µl and the IC50 value was determined to be 38.888µl.

Table 2: Percentage scavenging activity of ethanolic extract Zea mays cob (EEZM) determined by DPPH assay.

S. No	Volume of sample (µl)	Control Abs at 517nm	Sample Abs at 517nm	% scavenging	IC 50
1	10	0.213	0.183	14.08450704	38.88816856
2	20	0.213	0.151	29.10798122	
3	30	0.213	0.127	40.37558685	
4	40	0.213	0.101	52.58215962	
5	50	0.213	0.083	61.03286385	

Figure 2: Percentage scavenging activity of ethanolic extracts Zea mays cob (EEZM)



DISCUSSION

The advantages of finding phytobioconstituents are not restricted to the pharmaceutical industry; they also apply to the health-care industry, which makes nutritional supplements and other goods to support overall health, as well as the cosmetic industry. In terms of academia, this research opens up new options to study plants for their different contents, as well as increased awareness and knowledge of traditional applications. It also contributes to the scientific validation of traditional uses and opens up the possibility of combining current data with traditional formulation to generate unique, safe, and effective medications. The significant antioxidant activity of vitamin E, arachidonoids, and triterpene found in this plant's n-hexane extract will aid in reducing or delaying the effects of oxidative stress. More research is needed to prove the bioactive compound's efficacy, which will be the focus of our future research.

The antioxidant activity was evaluated using the DPPH (diphenylpicrylhydrazyl) test. DPPH is a stable free radical molecule with an oxidised absorbance of around 517nm. The DPPH assay is a simple and quick way to assess free radical scavenging activity. To generate a stable diamagnetic molecule, DPPH can take an electron or hydrogen radical. A decrease in DPPH radical absorption is indicated by a change in colour from purple to yellow. This shows how the antioxidant in a mixed solution interacts with the free radicals. The percentage of inhibition (Table 2) was calculated in this investigation to determine the antioxidant activity of the extracts and their ability to inhibit free radicals. Five distinct quantities of ethanolic extract of Zea mays cob (10, 20, 30, 40, and 50) showed different percentages of inhibition. Surprisingly, the extract's scavenging activity rose in proportion to its concentration. The antioxidant activity of the 50 µl extract was the highest. The IC50 value was determined by calculating the sample concentration required to inhibit 50% of radicals. The

antioxidant activity of samples increases as the IC50 value decreases. Antioxidants are one of the most important components of modern medicine because they reduce in vivo oxidative damage. Natural antioxidants can be found in abundance in plants. The physiologically active phytoconstituents present may thus be connected to the DPPH scavenging activity of the plant extracts.

CONCLUSION

Ethanolic extract of Zea mays cob exhibited potential antioxidant activity. It acts in a concentration-dependent manner. Ethanolic extract of Zea mays cob exhibited antioxidant activity because of presence of various phytoconstituents.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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