

Pharmacognostical Characterization and Evaluation of Invitro Antioxidant activity of *Daucus carota* and *Aloe barbadensis*

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ABSTRACT

Aloe barbadensis, a perennial, drought-resistant plant, is known as the healing plant due to its wound and burn healing properties. It has been used in health, beauty, medicine, and skin care for centuries. The pharmacologically active ingredients in aloe are concentrated in inner parenchymatous tissue called aloe gel and outer pericyclic tubules called aloe sap or aloe juice. These bioactive compounds are effective in treating various conditions, including burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentery, diarrhea, piles, and digestive system inflammatory conditions. *Aloe vera* is also used in cosmetic products to provide a healthy, supple skin look, reduce wrinkles, cure acne, rejuvenate, and give it a youthful glow. *Daucus carota*, also known as carrot, belongs to the Apiaceae or Umbelliferae family and is classified into twelve subspecies. The plant is classified into two groups, eucarota and gummiferi, each classified into five more subspecies. The chemical composition of different subspecies reveals the abundance of terpenes, phenolics, and flavonoids. The wild carrot was traditionally used for medicinal purposes by ancient Greeks and Romans, with its antioxidant, anticancer, anti-inflammatory, gastroprotective, hepatoprotective, antibacterial, and antifungal activities confirmed in-vitro and in-vivo over the past two decades. In present study, pharmacognostical examination is done prior to its preclinical activity in order to check for its purity and having a record of possible phytochemicals present in the plants which may be responsible for its pharmacological action. Recent research on MEAV and MEDC reveals its potent antioxidant capacity due to its high flavonoid content, which reduces damaging radicals. The presence of polyphenols in MEAV and MEDC results in reduced oxidative stress indicators and increased protective capacity of antioxidants. Both *Daucus carota* (carrot) and *Aloe barbadensis* (*Aloe vera*) demonstrate significant in vitro antioxidant activity, primarily due to the presence of phytochemicals like flavonoids, carotenoids, and polyphenols. *Daucus carota* exhibits strong scavenging of free radicals, while *Aloe barbadensis* shows potent DPPH scavenging and reducing power. Extraction methods and plant parts influence the concentration and activity of these antioxidants.

Keywords: *Aloe barbadensis*; *Daucus carota*; Methanol Extract; Pharmacognostical Examination; Antioxidants.

1. INTRODUCTION

Aloe barbadensis, a perennial, drought-resistant plant, is known as the healing plant due to its wound and burn healing properties. It has been used in health, beauty, medicine, and skin care for centuries, with significant roles in indigenous systems like ayurveda, siddha, unani, and homoeopathy. The pharmacologically active ingredients in aloe are concentrated in inner parenchymatous tissue called aloe gel and outer pericyclic tubules called aloe sap or aloe juice. These bioactive compounds are effective in treating various conditions, including burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid indigestion, ulcers, diabetes, skin diseases, dysentery, diarrhea, piles, and digestive system inflammatory conditions. *Aloe vera* is also used in cosmetic products to provide a healthy, supple skin look, reduce wrinkles, cure acne, rejuvenate, and give it a youthful glow (1-5). *Daucus carota*, commonly known as carrot, belongs to the Apiaceae or Umbelliferae family and is part of the *Daucus* genus. The plant is classified into twelve subspecies, with the most well-known being *D. carota* ssp. *sativus* and *D. carota* ssp. *boissieri* (red carrot). Differentiation among *D. carota* L. subspecies is difficult due to the coexistence of cultivated and wild carrot samples. The chemical composition of plant extracts differs between multiple subspecies and among plants of the same subspecies (6-10).

In order to develop a successful antidiabetic medication, it is necessary to identify and characterise the chemical components of *Aloe barbadensis* and *Daucus carota* as part of the pharmacognostic evaluation. Clinical and preclinical trials are needed to evaluate the safety and effectiveness of *Aloe barbadensis* and *Daucus carota* extract as antidiabetic agents. If the plant is to be believed as a natural solution for diabetes management, these studies are vital.

2. MATERIALS AND METHODS

2.1 Collection and Identification of the Plant Material

The *Daucus carota* and *Aloe vera* were collected in Feb. 2024 from the nearby areas of college campus, Bhopal. The voucher specimen was submitted to the Herbarium and Museum Section of the Institute.

2.2 Processing and Preparation of the methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV)

The fruits were dried, ground into a coarse powder, and defatted to remove wax and lipids. The powder was extracted using methanol and refluxed with petroleum ether. The defatted marc was soaked in purified water for 48 hours, then filtered through Whatman filter paper. The filtrate was dried in a rotary evaporator, and the dried residue was used as a crude extract for further research. The abbreviations used for methanolic extracts of *Daucus carota* and *Aloe vera* are MEDC and MEAV, respectively. The percentage yield was calculated and quantitative (11) and qualitative (12) phytochemical evaluation was done for the extract.

2.3 Metabolic profiling of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) by GC-MS analysis

The study involved analyzing the content of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) using GC-MS. The Methanolic extract was suspended in a methoxylamine hydrochloride solution and GC grade pyridine. The lipid content was analyzed using Thermo Trace GC Ultra coupled with Thermo fisher DSQ II mass spectrometers. Chromatographic separations of metabolites were performed on a 30 m x 0.25 mm Thermo TR50 column. Xcalibur software was used to process the data. The GC oven temperature was maintained at 70°C for 5 minutes, then raised to 290°C. The sample was injected in split mode with helium as a carrier gas. The resulting GC-MS profile was analyzed using Replib, WILLY, and NIST mass spectral libraries (13). The concentration of metabolites was calculated on the percent peak area basis.

2.4 Evaluation of Invitro Anti Oxidant Activity

2.4.1 DPPH scavenging assay

This investigation involves a process of dissolved DPPH in methanol to create a stock solution with a concentration of 0.1 mM. The methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) is then diluted with methanol to create different concentrations. Each test tube receives 3 mL of the stock solution, and the mixture is incubated at room temperature for half an hour. A spectrophotometer is used to test the absorbance at 517 nm, indicating higher antioxidant activity (14).

2.4.2 Reducing power assay

This investigation involves creating a stock solution of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) at varying concentrations, preparing dilutions, and combining phosphate buffer, potassium ferricyanide, and the test chemical solution. The reaction mixture is incubated for 20 minutes at 50°C, then centrifuged to remove precipitated proteins. Distilled water and ferric chloride solution are then added to the supernatant. The complex's absorbance at 700 nm is determined using spectrophotometric measurement (15).

3. RESULTS AND DISCUSSION

3.1 Standardization of the Crude Drug

The physicochemical constants of *Daucus carota* and *Aloe vera* were found to be as mentioned in the Table 1. The physicochemical studies viz. ash content, extractive value, moisture content, pH indicated that the *Daucus carota* and *Aloe vera* are of standard quality.

Table 1: Physicochemical evaluation of *Daucus carota* and *Aloe vera*

Sr. No.	Standardization parameters	Value %w/w	
01	Ash analysis		
	❖ Ash Content (Total Ash)	19.03 ± 0.021	18.96 ± 0.301
	❖ Acid In-Soluble Ash	1.003 ± 0.005	0.941 ± 0.714
02	Extractive value (Maceration Process)		
	❖ Alcohol soluble	10.104 ± 0.308	11.548 ± 0.432
	❖ Water soluble	38.41 ± 0.415	31.11 ± 0.010

03	Moisture content (Loss On Drying)	10.500 ± 0.172	9.096 ± 0.032
04	pH (1% aqueous solution)	6.990 ± 0.152	6.999 ± 0.528

3.2 Percentage (%) Yield

The methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV) were found to be yellowish green and greenish blue with sticky consistency was obtained with percent yield of 53.69 % and 61.44 % w/w, respectively.

3.3 Phytochemical Screening and Quantitative Estimation

MEDC and MEAV showed presence of carbohydrates, proteins, amino acids, alkaloids, saponins, sterols, tannins and phenolic compounds flavonoids. The total flavonoid content of MEDC and MEAV were found to be 20.71 ± 0.041 and 27.45 ± 0.471 mg quercetin equivalents/g of extract and total phenolic content of MEDC and MEAV were found to be 24.58 ± 0.107 and 45.08 ± 0.358 mg tannic acid equivalents/g of extract.

3.4 GC-MS Analysis of methanolic extracts of *Daucus carota* and *Aloe vera* (MEDC and MEAV)

The present study annotated 31 compounds from the *Daucus carota* methanolic extract via UPLC-QTOF-MS/MS (Table 2; Figure 1). The GC analysis of MEAV resulted in 25 significant retention time (RT) peaks which on the interpretation by a Mass Spectrometer (MS) revealed more than 200 phytochemical compounds (approximately 20 compounds/peak) present in the MEAV. In the GC-MS analysis, 25 bioactive compounds, based on their peak area percentage, molecular weight and molecular formula were identified in the methanolic extract of *Aloe vera* (Table 3; Figure 2).

Table 2: GC-MS Analysis of Methanol Extract of *Daucus carota*

S.N.	Identified Compound	M.F.	M.wt.	Rt
1.	Malic acid	C4H6O5	134	3.11
2.	Maleic acid	C4H4O4	116	3.14
3.	3-Hydroxybenzoicacid	C7H6O3	138	10.18
4.	Vanillic acid	C8H8O4	168	10.64
5.	Caffeicacid	C9H8O4	180	11.06
6.	Neochlorogenicacid	C16H18O9	354	11.12
7.	1,5-Dicaffeoylquinicacid	C25H24O12	516	11.16
8.	3,5-Di-O-caffeoylquinicacid	C25H24O12	516	10.92
9.	Pyrocatechol	C6H6O2	110	11.22
10.	Esculin	C15H16O9	340	11.34
11.	Isoferulicacid	C10H10O4	194	11.40
12.	o-Coumaricacid	C9H8O3	164	11.62
13.	4-O-Caffeoylquinicacid	C16H18O9	354	11.70
14.	FrangulinA	C21H20O9	416	11.91
15.	1,3-O-dicaffeoylquinicacid(Cynarin)	C25H24O12	516	12.10
16.	Luteolin-7,3'-di-O-glucoside	C27H30O16	610	12.12
17.	Protocatechuicaldehyde	C7H6O3	138	12.14
18.	Datisctin3-O-rutinoside(Datisctin)	C27H30O15	594	12.48
19.	7,8-Dihydroxycoumarin(Daphnetin)	C9H6O4	178	12.51
20.	Scutellarin	C21H18O12	462	12.81
21.	Luteolin7-glucoside	C21H20O11	448	12.83

22.	Luteolin-4'-O-glucoside	C21H20O11	448	12.84
23.	Diosmin	C28H32O15	608	12.99
24.	3-Hydroxybenzaldehyde	C7H6O2	122	13.34
25.	Apigenin7-glucoside	C21H20O10	432	13.36
26.	Tectoridin	C22H22O11	462	13.42
27.	4,5-Dicaffeoylquinicacid	C25H24O12	516	13.47
28.	Icariside	C27H30O10	530	14.30
29.	7,8,3',4'-Tetrahydroxyflavone	C15H10O6	286	14.97
30.	Apigenin	C15H10O5	270	15.84
31.	Chrysoeriol	C16H12O6	300	16.03

Table 3: GC-MS Analysis of Methanol Extract of *Aloe vera*

S.N.	Identified Compound	M.F.	M.wt.	Rt
1.	HexanoicAcid,2-Acetyl-,Ethyl Ester	C10H18O3	186.25	8.74
2.	OctanoicAcid,2- Hexyl-	C14H28O2	228.37	8.74
3.	5- Hydroxymethyl-furfural/ 1,2,4- Benzenetriol	C ₆ H ₆ O ₃	126.11	10.1
4.	4- Mercaptophenol	C ₆ H ₆ O ₅	158.11	10.1
5.	5-Acetoxymethyl-2-furaldehyde	C ₈ H ₈ O ₄	168.15	10.1
6.	4,5-Dimethyl-4-hexene-3-one	C ₈ H ₁₄ O	126.2	10.1
7.	Alpha-D- Glucose/Epi- inositol/D-Allose/	C6H12O6	180.16	13.47
8.	Lactose/Malt- ose/Trehalose	C12H22O11	342.3	13.47
9.	TridecanoicAcid	C13H26O2	214.34	13.47
10.	Maltotriose	C18H32O16	504.4	13.47
11.	Methylá-d- galactopyra-noside	C7H14O6	194.18	13.47
12.	CitronellylButyrate	C14H26O2	226.35	16.73
13.	ButanoicAcid,3- Llethyl-, 3,7- Diliethyl-6- OctenylEster/ CitronellylIso- valerate	C15H28O2	240.38	16.73
14.	PhytolAcetate	C22H42O2	338.6	16.37
15.	Phytol/3,7,11,15-Tetramethyl-2- Hexadecen-1-Ol	C20H40O	265.5	16.37
16.	Octadecanol	C18H38O	270.5	16.37
17.	MyristicAcid	C14H28O2	228.37	17.4
18.	N-DecanoicAcid/Capricacid	C10H20O2	172.26	17.4

19.	PentadecanoicAcid	C15H30O2	242.4	17.4
20.	N-Hexadecanoic Acid/Palmitic acid	C16H32O2	256.42	17.4
21.	Octadecanoic Acid/Stearic acid	C18H36O2	284.5	17.4
22.	TetracosanoicAcid	C24H48O2	368.6	17.4
23.	9,12,15-Octadecatrienoic Acid, Methyl Ester, (Z,Z,Z)-	C19H32O2	292.5	18.82
24.	Palmitic anhydride	C32H62O3	494.5	21.33
25.	Glyceryl 1,3- Dipalmitate	C35H68O5	568.1	21.33

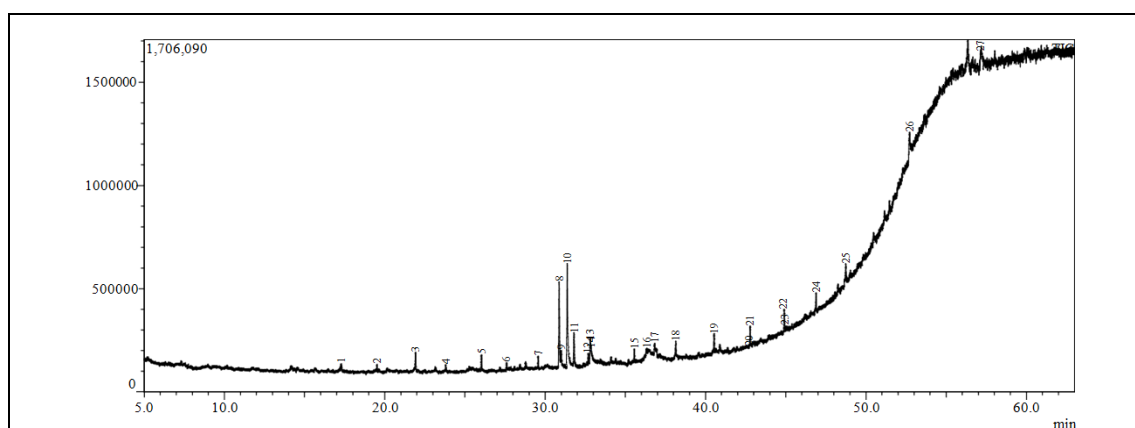


Figure 1: GC-MS Chromatogram of Methanol Extract of *Daucus carota*

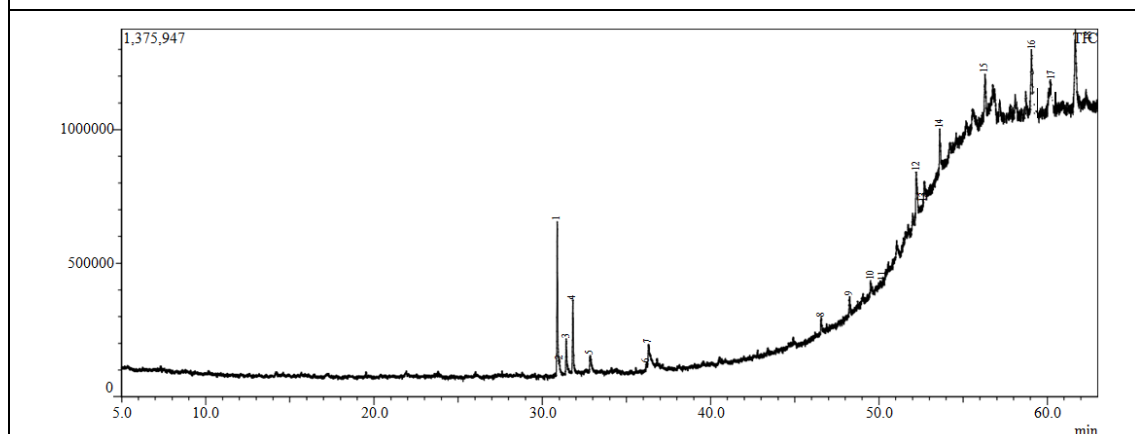


Figure 2: GC-MS Chromatogram of Methanol Extract of *Aloe vera*

3.5 Assessment of Antioxidant Activity

3.5.1. DPPH scavenging assay

The MEDC and MEAV in concentration range of 100-1000 µg/ml inhibited DPPH radical formation as indicated by concentration dependent decrease in the purple colour of the solution. Similar effect was obtained with standard antioxidant- Butylated Hydroxy Toluene (BHT) in the concentration range of 10-100 µg/ml. In linear regression analysis of concentration versus percent DPPH inhibition was carried out. The linear regression coefficient of MEDC, MEAV and BHT were $r^2=0.998$, $r^2=0.992$ and $r^2=0.998$, respectively, suggesting that the DPPH scavenging was concentration dependent. The IC_{50} value of MEDC, MEAV and BHT, obtained from regression analysis, were 602.733, 399.408 and 50.173 µg/ml, respectively (Table 4).

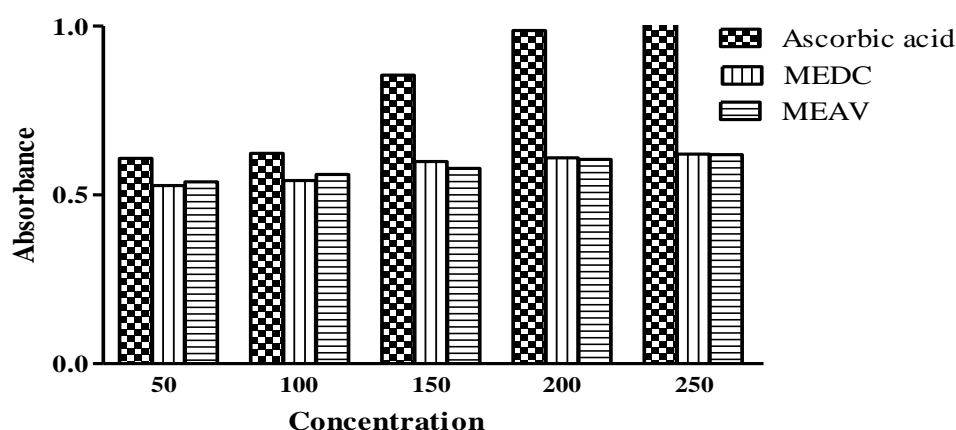
Table 4: Effect of MEDC and MEAV on DPPH radical scavenging

Concentration (µg/ml)		% DPPH Inhibition	IC ₅₀ Value
MEDC	100	16.357±0.315	599.807 µg/ml
	200	27.293±0.173	
	400	37.545±0.990	
	600	48.515±0.285	
	800	62.749±0.824	
	1000	75.064±0.223	
MEAV	100	21.25±0.145	403.108 µg/ml
	200	39.14±0.845	
	400	51.09±1.154	
	600	69.45±0.425	
	800	80.09±0.666	
	1000	95.14±0.856	
BHT	10	13.311±0.397	50.173 µg/ml
	20	25.706±0.529	
	40	47.305±0.496	
	60	65.163±0.636	
	80	75.064±0.223	
	100	80.271±0.257	

(Values are mean± SEM; n=3; IC₅₀= 50% Inhibitory concentration)

3.5.2. Reducing power assay

The MEDC and MEAV in the concentration range of 50-250 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green colour absorbance measured at 700 nm. Similar effect was obtained with standard antioxidant- ascorbic acid in the concentration range of 50-250 µg/ml. A concentration verses absorbance graph comparing ascorbic acid and MEDC and MEAV were plotted and depicted in (Figure 3).

**Figure 3: Effect of MEDC and MEAV on reducing potential**

(Results are expressed as Mean \pm SEM; n=3)

4. CONCLUSION

The presence of polyphenols in MEAV and MEDC results in reduced oxidative stress indicators and increased protective capacity of antioxidants. Both *Daucus carota* (carrot) and *Aloe barbadensis* (*Aloe vera*) demonstrate significant in vitro antioxidant activity, primarily due to the presence of phytochemicals like flavonoids and polyphenols. *Daucus carota* exhibits strong scavenging of free radicals, while *Aloe barbadensis* shows potent DPPH scavenging and reducing power. Extraction methods and plant parts influence the concentration and activity of these antioxidants.

5. CONFLICT OF INTEREST

None

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