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Phytoconstituents Analysis of Ornamental Plants Using Gc-Ms And Hptlc

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ABSTRACT

In this current research work two ornamental plants collected, extracted and study their phytoconstituents analysis. The phytochemical constituents were analyzed using Gas Chromatography-Mass Spectrometry and HPTLC Densitometric chromatogram of Standard (Quercetin) and hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* aerial parts. Additionally, the total polyphenolic content was determined by calculating it as gallic acid, while the total flavonoid content was determined by calculating it as Quercetin using the UV technique. *A. obesum* exhibits a significant amount of total flavonoids while *G. rigens* demonstrates a substantial amount of total polyphenols. The hydroalcoholic extracts of *Adenium obesum* comprised a minimal quantity of quercetin, as shown by our results. Both plants have a variety of phytoconstituents which may possess various pharmacological activity and its α-glucosidase and antioxidant levels may support its use in traditional medicine, particularly for reducing diabetes-related problems.

Keywords: Phytoconstituents, HPTLC, Gas Chromatography-Mass Spectrometry, Quercetin, α-glucosidase, *Adenium obesum, Gazania rigens.*

INTRODUCTION

Herbal medications include a high concentration of phytochemicals, which are thought to have a significant impact on the treatment of chronic illnesses like cancer, diabetes, and coronary heart disease.[1] Therefore, it is imperative to conduct a thorough investigation of traditional plants using phytochemical methods in order to identify and extract bioactive compounds. This will enable the development of novel, safer, and more effective medications derived from natural sources. Currently, there is a significant focus on the use of medicinal plants and their derivatives for the treatment of various infectious diseases.[2] The present study involved the preparation of hydroalcoholic extracts from *Adenium obesum* and *Gazania rigens*, followed by a comprehensive investigation of the quantitative phytochemical analysis of both extracts. The primary bioactive compounds responsible for diverse activities include alkaloids, tannins, terpenoids, flavonoids, phenols, and glycosides.[3, 4] The number of studies on medicinal plants from various regions of the world is rapidly growing on daily basis.[5] The World Health Organization has claimed that more than 80% of the global population utilizes plant extracts, plant products, and active chemical substances to cure various infectious diseases.[6,7] Plants and plant-based products have long been significant in both preventing and treating human diseases, whether curable or incurable.[8] The majority of countries rely on traditional medicine as their main healthcare system.[9, 10] This study has conducted extensive research on ornamental plant extracts and their phytoconstituents may possess efficacy against various disorders.[11, 12]

MATERIALS AND METHODS

The aerial portions of *Adenium obesum* and *Gazania rigens* were obtained from diverse places, specifically

Jodhpur and Jaipur. The authentication of obtained dried aerial portions of *Adenium obesum* and *Gazania rigens* were confirmed by Botanical Survey of India, Jodhpur with reference no. – BSI/AZRC/L12012/ Tech/2019-20 (Pl. Id).

2.1 Preparation of Extracts

Dried powdered aerial parts of *Adenium obesum* and *Gazania rigens* (250 gm each) were separately extracting exhaustively with mixture of ethanol and water (1:1) in a Soxhlet apparatus to obtain hydroalcoholic extracts. The hydroalcoholic extracts were concentrated using a rotating vacuum evaporator, and all of the hydroalcoholic extracts were stored in a vacuum desiccator for subsequent quantitative analysis. [13, 14]

1.2 Quantification of Total Phenolic Content

The overall phenol concentration in the aerial parts of *Adenium obesum* and *Gazania rigens* was determined using the standard technique.[15]

Preparation of Standard Curve

In 100 ml of 50% ethanol, gallic acid (10 mg) was dissolved and then dilutions were made, i.e., 30, 60, 90, 120, 150 or 180 µg/ml. Each dilution was aliquoted (1 ml) into a test tube and subsequently stirred with 10 ml of purified water. After that, it was let to incubate at ambient temperature for 5 minutes immediately following the introduction of 1.5 ml of Folin Ciocalteu's reagent. Each test tube was supplemented with 4 ml of a sodium carbonate solution with a concentration of 20% (w/v) and then adjusted volume to 25 ml using distilled water. It was stirred vigorously and was allowed to rest at ambient temperature for 30 minutes. The absorbance of the standard was measured relative to blank, specifically distilled water, using a UV/VISIBLE spectrophotometer at a wavelength of 765 nm.

Preparation of Test Samples

Each hydroalcoholic extract of the aerial portions of *Adenium obesum* and *Gazania rigens*, weighing 250 mg each, was separately added to 15 ml of 50% ethanol. The mixture was then subjected to maceration three times for duration of 2 hours each. Subsequently, the mixture passed filtration and the volume was precisely modified to 25 ml by adding a 50% ethanol solution in a volumetric container. For each dilution, a 1 ml sample was obtained and subsequently mixed with 10 ml of distilled water to make a dilution in test tube. Afterwards, it was let to incubate at ambient temperature for 5 minutes immediately following the introduction of 1.5 ml of Folin Ciocalteu's reagent. Each test tube was supplemented with 4 ml of a sodium carbonate solution with a concentration of 20% (w/v) and then adjusted volume to 10 ml using distilled water. It was stirred vigorously and was allowed to rest at ambient temperature for 30 minutes. The absorbance of the sample at 765 nm was measured relative to blank, specifically distilled water. The absorbance of the standard compared to blank, specifically distilled water, was analyzed using a UV/VISIBLE spectrophotometer at 765 nm.

Quantification of Total Phenol Content

The gallic acid standard curve was utilized to quantify the total phenol concentration. A calibration curve plotting the absorbance against the concentration of gallic acid was generated. The findings were computed using the given formula and presented as a percentage w/w (mean ± standard deviation).

$$\text{Total phenolic content (\% w/w)} = GAE \times V \times D \times 10^{-6} \times 100 / W$$

Where GAE = gallic acid equivalents (µg/ml); V = total volume of sample (ml); D = dilution factor; W = sample weight (g).

2.3 Quantification of Total Flavonoid Content

The overall flavonoid concentration in the aerial portions of *Adenium obesum* and *Gazania rigens* was

determined using a standardized technique.[16]

Preparation of Standard Curve

A solution was prepared by dissolving 20 mg of quercetin in 100 ml of ethanol i.e., 200 µg/ml, and then the dilutions were prepared to 20, 40, 60, 80, 100 or 120 µg/ml. The standard solutions (0.5 ml) were each combined with 1.5 ml of ethanol (95%), 0.1 ml of aluminium chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. This mixture was rest at ambient temperature for 30 min. The determination of the reaction mixture's absorbance was conducted using a UV/VIS spectrophotometer at a specific wavelength of 415 nm. The distilled water in blank was used for the substitution of the aluminium chloride (10%) in same amount.

Preparation of Test Samples

In 25 ml of ethanol, 250 mg of each hydroalcoholic extract of aerial parts of *Adenium obesum* and *Gazania rigens* were dissolved separately. The test samples (0.5 ml) were individually combined with 1.5 ml of ethanol (95%), 0.1 ml of aluminium chloride (10%), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water for the determination of flavonoids content. The distilled water in blank was used for the substitution of the aluminium chloride (10%) in same amount.

Quantification of Total Flavonoid Content

The standard curve of quercetin was used for the quantification of total flavonoids content. A calibration curve was generated by plotting the absorbance values against the concentrations of quercetin. The findings were computed using the subsequent formula and were presented as percentage w/w (mean ± S.D.).

$$\text{Flavonoids content (\% w/w)} = QE \times V \times D \times 10^{-6} \times 100 / W$$

Where QE = quercetin equivalents (µg/ml); V = total volume of sample (ml); D = dilution factor; W = sample weight (g).

2.4 Quantification of Phytoconstituents of Aerial Parts of *Adenium obesum* and *Gazania rigens* using Gas Chromatography-Mass Spectrometry Analysis[17]

Gas Chromatography-Mass Spectrometry Conditions

The each hydroalcoholic extract of aerial parts of *Adenium obesum* and *Gazania rigens* was dissolved separately in isopropyl liquor to acquire a last grouping of 30 mg/ml and went through Whatman polytetrafluoroethylene syringe channel of pore size 0.22 µ for GC-MS investigation. Helium was utilized as bearer gas and all out run time was of 24 min. Splitless infusion mode was chosen and infusion volume was set at 1 µl. Chamber temperature was set at 120º C for 7 min and expanded to 200º C at 20º C/min and held for 17 min. The injector temperature was 230º C.

2.5 Quantitative Determination of Quercetin Aerial Parts of *Adenium obesum* and *Gazania rigens* using HPTLC Densitometry[18]

Formulation of marker compound stock solution

The marker component stock solution was made by properly dissolving 1 mg of quercetin in 1 ml of methanol.

Preparation of Standard Plot

To prepare three dilutions of varying concentration of marker component (2, 4 and 6 µg/ml), the stock solution was diluted using methanol as a solvent. Every dilution (10 µl) of different concentration was loaded on precoated plates. The solvent system used to develop the plate was – toluene: ethyl acetate: glacial acetic acid (6:3:1). The TLC plate that was prepared for marker component was analyzed at 254 nm. The area under curve of the peak matching to the marker compound was recorded for each track.

Determination of Marker Component

In triplicate, a 10 µl test solution of each hydroalcoholic extract from the aerial portions of *Adenium obesum* and *Gazania rigens* was put to a pre-coated plate measuring 5 × 10 cm. The plate was developed using a solvent system consisting of toluene, ethyl acetate, and glacial acetic acid at a ratio of 6:3:1. The TLC plate that was prepared for the marker compound was analyzed at a wavelength of 254 nm. The test sample was analyzed by scanning the average area under curve of the peak relating to the marker component at a wavelength of 254 nm. The concentration of the marker component was then determined using the regression equation derived from the standard plot.

RESULTS

3.1 Determination of Total Phenols and Flavonoids Content

Figure 1 indicates Calibration curve of gallic acid *vs* absorbance. Figure 2 indicates standard curve plots of quercetin *vs* absorbance. Figure 3 and 4 shows a chromatogram of chemical compounds present in hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* aerial parts identified by GC and GC–MS analysis. A total of 37 phytoconstituents were identified in hydroalcoholic extracts of *Adenium obesum* and 26 phytoconstituents in hydroalcoholic extracts of *Gazania rigens* by GC and GC–MS analysis (Table 2 and 3). Quercetin amount, spectra overlay and TLC fingerprinting identified in TLC Densitometric analysis (Table 4, Figure 5, 6 and 7)

Table 1: The results of total phenols and flavonoids contents in hydroalcoholic extract of *Adenium obesum* and *Gazania rigens* aerial parts

Plant material	Total phenols content (% w/w) Mean^n±S.D.	Total flavonoids content (% w/w) Mean^n±S.D.
Adenium obesum	2.45 ± 0.25	5.25 ± 0.11
Gazania rigens	4.18 ± 0.15	3.92 ± 0.46

n=3

3.2 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chromatogram of hydroalcoholic extracts of *Adenium obesum* aerial parts showed thirty seven peaks each representing a different phytoconstituent.

Table 2: Phytoconstituents present in hydroalcoholic extract of *Adenium obesum* aerial parts identified by GC-MS.

Peak#	R.Time	Area%	Name
1	5.921	33.27	Tetraethyl silicate
2	6.223	18.99	Silane, diethoxydimethoxy-

3	8.953	1.04	1,4-Dioxane-2,6-dimethanol
4	9.039	2.14	Hexanoic acid, dodecyl ester
5	9.691	0.46	2-Cyclopenten-1-one, 3-methyl-
6	9.77	0.64	Ethanol, 2-phenoxy-
7	9.926	0.35	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-
8	10.67	0.33	Cyclooctane, 1,2-diethyl-
9	11.75	0.41	2(3H)-Furanone, dihydro-5-pentyl-
10	11.84	0.48	Cyclopentane, 1-pentyl-2-propyl-
11	12.149	0.64	3-Hexadecene, (Z)-
12	13.22	0.62	1-Dodecanol
13	13.315	0.24	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-
14	13.637	0.83	7,9-Di-tertbutyl-1-oxaspiro[4,5]deca-6,9-dien-
15	14.062	0.49	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4
16	14.636	5.19	Diethyl Phthalate
17	14.759	0.84	Nonadecane, 9-methyl-
18	15.059	0.25	Cyclooctasiloxane, hexadecamethyl-
19	15.146	0.23	Megastigmatrienone
20	15.303	0.28	Pentadecane, 2,6,10-trimethyl-
21	15.495	0.33	2-Methyltetracosane
22	15.591	1.03	8-Pentadecanone
23	15.912	2.65	Heptadecane

24	15.948	2.47	3,4-dihydroxyphenl- 5,7 dihydroxy 4H-1-benzopyran-4-one
25	16.613	0.62	Dotriacontane, 1-iodo-
26	16.696	0.55	Heptadecane, 3-methyl-
27	16.932	0.36	Pentadecafluorooctanoic acid, hexadecyl ester
28	17.006	3.83	Eicosane
29	17.446	0.76	2-Pentadecanone, 6,10,14-trimethyl-
30	17.605	0.18	Dodecane, 4-methyl-
31	17.663	0.89	Phthalic acid, 2,2-dimethylpent-3-yl tetradecyl
32	17.762	0.9	2-Methylhexacosane
33	18.293	0.91	Hexadecanoic acid, methyl ester
34	18.627	0.87	1,2-Benzenedicarboxylic acid, butyl 2-ethylene
35	18.762	0.42	Nonyl tetradecyl ether
36	19.001	2.48	4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acr
37	20.004	1.15	Eicosane

The chromatogram of hydroalcoholic extract of *Gazania rigens* aerial parts showed twenty six peaks each representing a different phytoconstituent.

Table 3: Phytoconstituents present in hydroalcoholic extract of *Gazania rigens* aerial parts identified by GC-MS.

Peak#	**R.Time**	**Area%**	**Name**
1	5.909	22.95	Tetraethyl silicate
2	6.224	11.24	Silane, diethoxydimethoxy-
3	7.355	1.37	Ethanone, 1-(1H-pyrrol-2-yl)-

4	8.638	2.59	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
5	9.044	1.73	Cyclopentane, (1-methylethyl)-
6	9.309	0.79	Estragole
7	10.897	0.28	1-Octanol, 2-butyl-
8	12.15	0.43	3-Hexadecene, (Z)-
9	12.684	6.61	Caryophyllene
10	12.966	2.5	Nerolidol isobutyrate
11	13.375	2.16	1-Pentadecene
12	13.543	16.52	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl
13	13.709	1.27	.beta.-Bisabolene
14	14.32	1.48	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E
15	14.636	3.22	(3,4-dihydroxyphenyl)prop-2-enoyl, oxy 1,4,5-trihydroxycyclohexane-1-carboxylic acid
16	14.744	0.97	Caryophyllene oxide
17	15.061	0.39	Diethyl Phthalate
18	15.371	0.87	Caryophylla-4(12),8(13)-dien-5.alpha.-ol
19	15.912	9.7	Cryptomeridiol
20	16.76	0.13	Cyclononasiloxane, octadecamethyl-
21	17.597	0.58	Acetamide, N-(3,4,5-trimethoxyphenethyl)-
22	18.297	1.46	Hexadecanoic acid, methyl ester
23	18.48	3.73	2,4-Decadienamide, N-isobutyl-, (E,E)-
24	19.002	2.02	4-(3,5-Di-tert-butyl-4-hydroxyphenyl)butyl acr

25	19.945	0.37	cis-11,14-Eicosadienoic acid, methyl ester
26	20.004	0.74	9-Octadecen-12-ynoic acid, methyl ester

3.3 Quantitative Determination of Bioactive Constituent Quercetin[19]

A calibration curve was established between various concentration of quercetin and their corresponding peak regions (Figure 6) with scanning at 254 nm. The linearity of the standard graph of quercetin was established within the range of 100 to 600 ng. (Figure 5). The substance of quercetin in hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* aerial parts was determined utilizing regression equation of standard graph. The result of quantification of quercetin in hydroalcoholic extracts of *Adenium obesum* aerial parts was presented in table 4.

Table 4: Percentage yield of quercetin in hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* aerial portions.

Plants	**Percentage yield (w/w) (Mean^n ± S.D.)**
Adenium obesum	0.00373 ± 0.00004
Gazania rigens	Not Detected

Determination of Total Phenols and Flavonoids Content

The results of determination of total phenols and flavonoids content of hydroalcoholic extract of *Adenium obesum* and *Gazania rigens* aerial parts are presented in table 1 and figures 1 and 2.

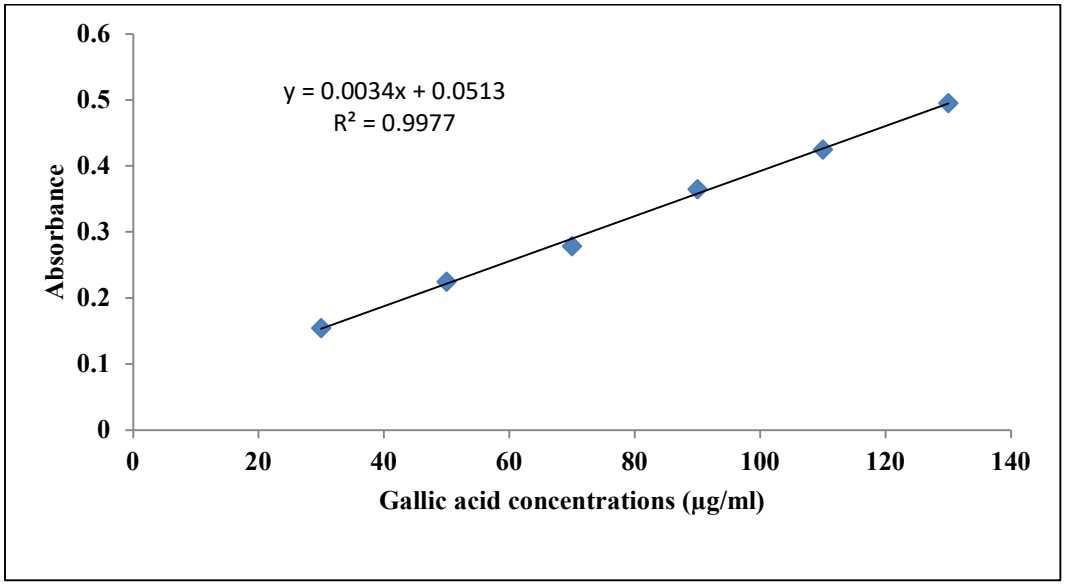


Figure 1: Calibration curve of gallic acid *vs* absorbance.

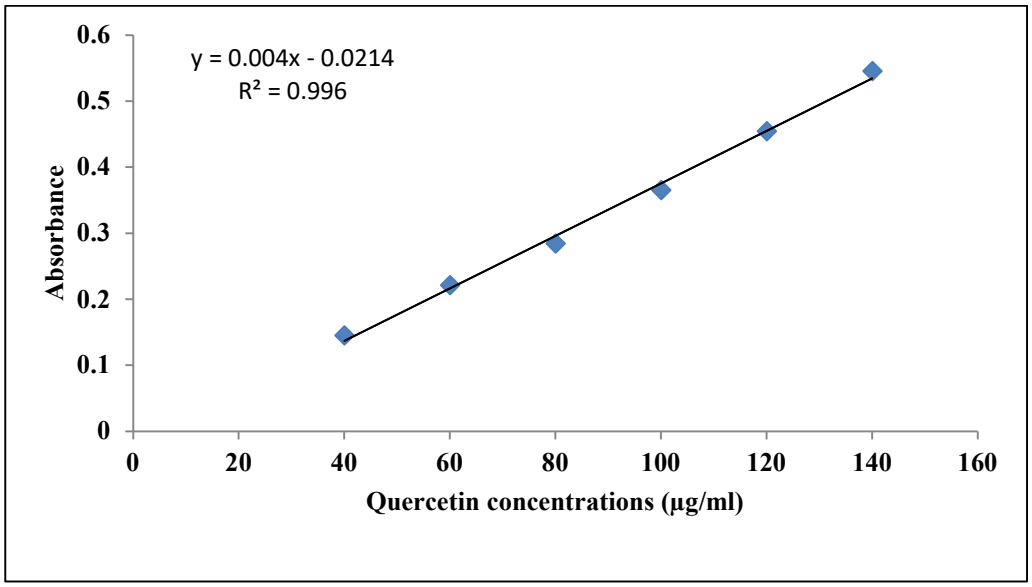


Figure 2: Standard curve plots of quercetin *vs* absorbance.

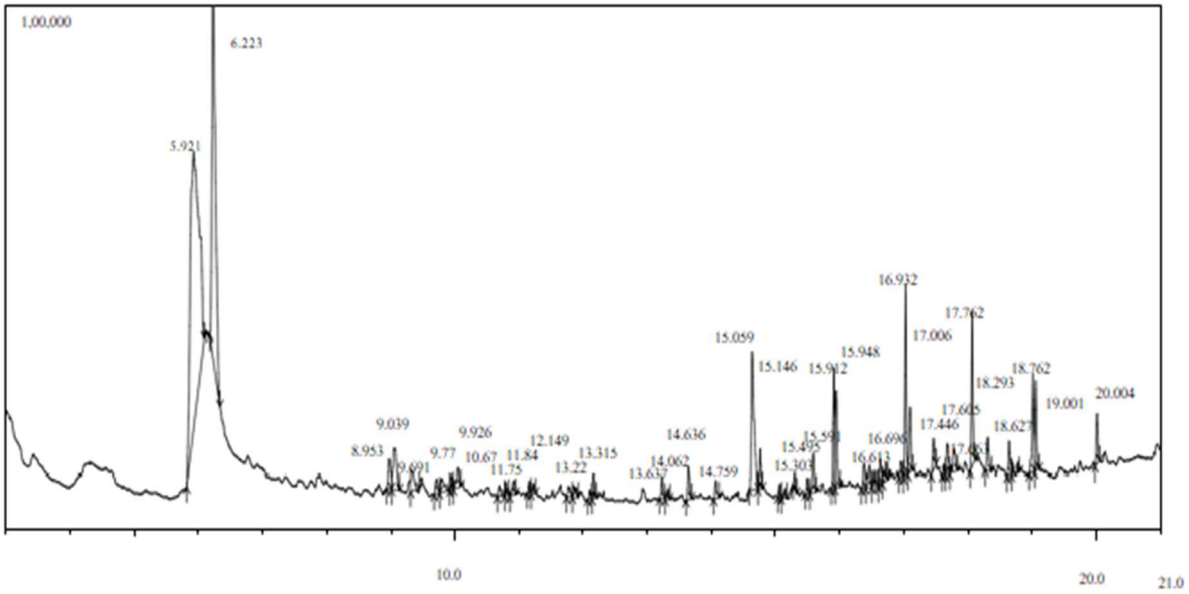


Figure 3: GC-MS chromatogram of hydroalcoholic extract of *Adenium obesum* aerial parts.

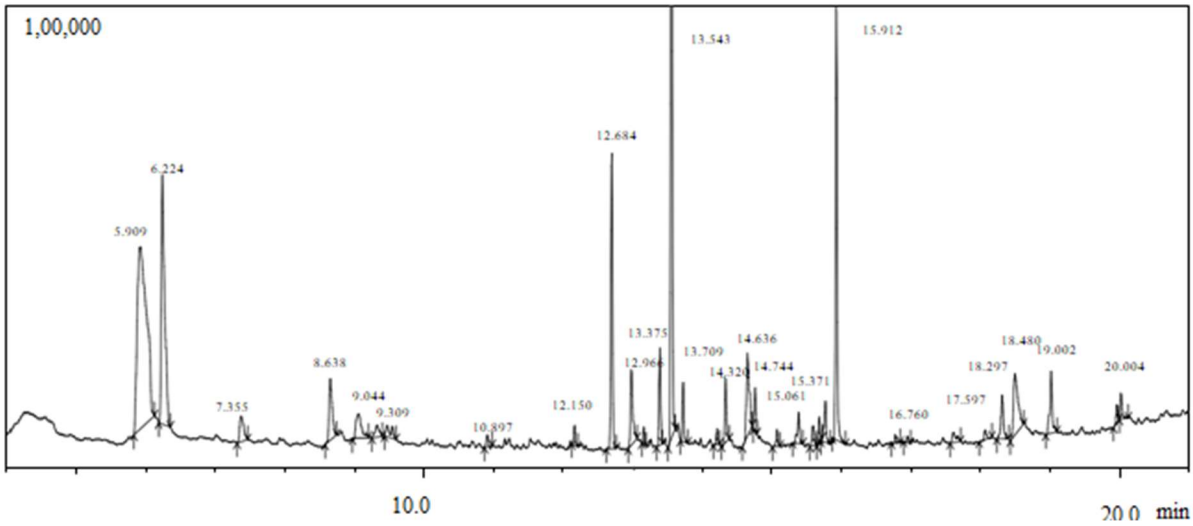


Figure 4: GC-MS chromatogram of hydroalcoholic extract of *Gazania rigens* aerial parts.

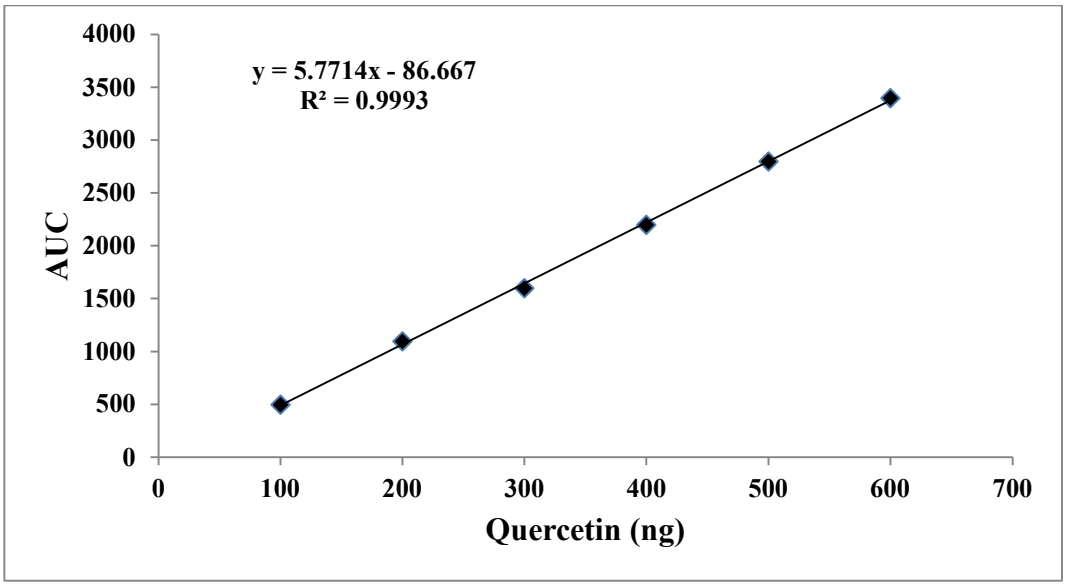


Figure 5: Standard plot of quercetin between area under curve and quercetin amount in TLC Densitometric analysis.

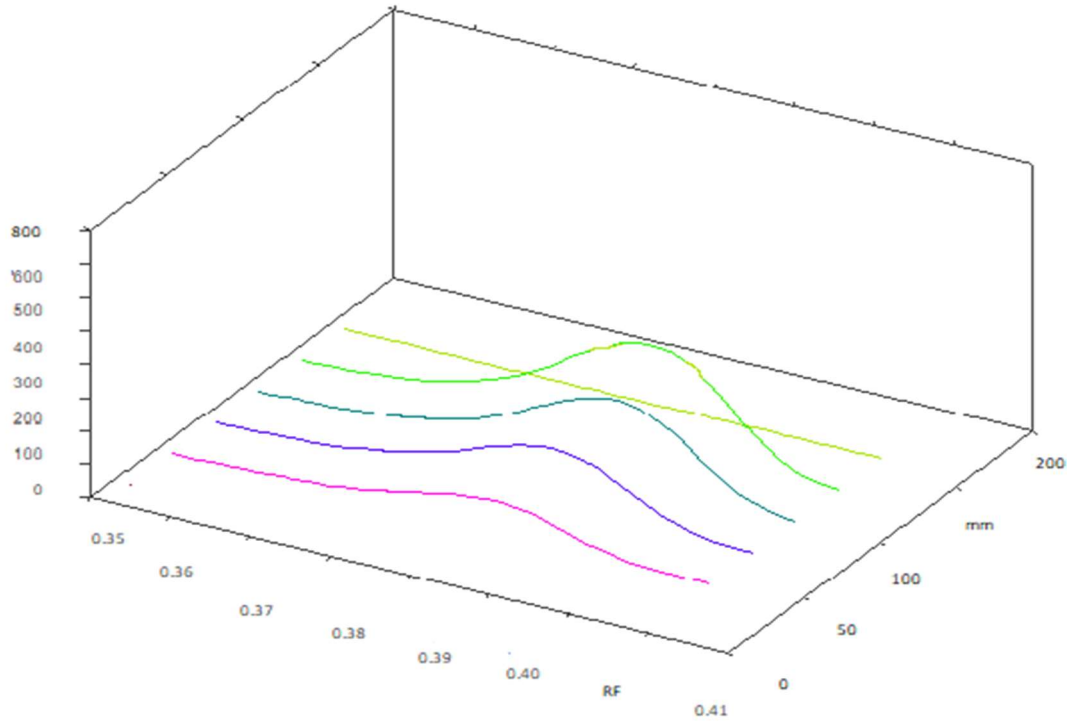
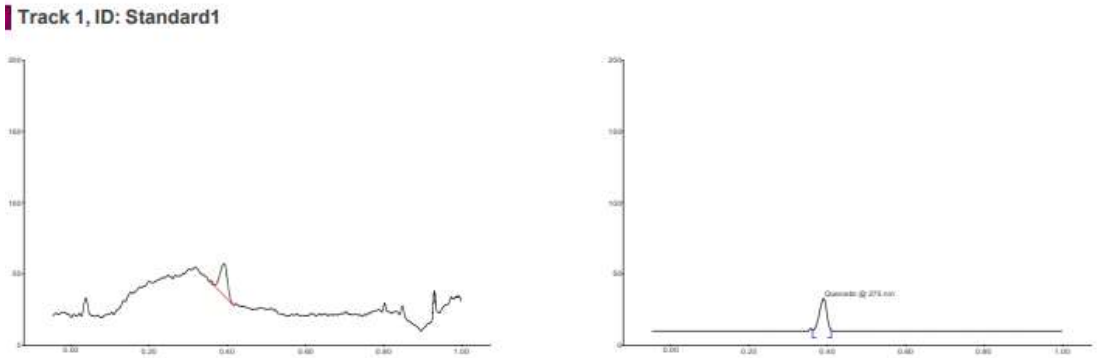
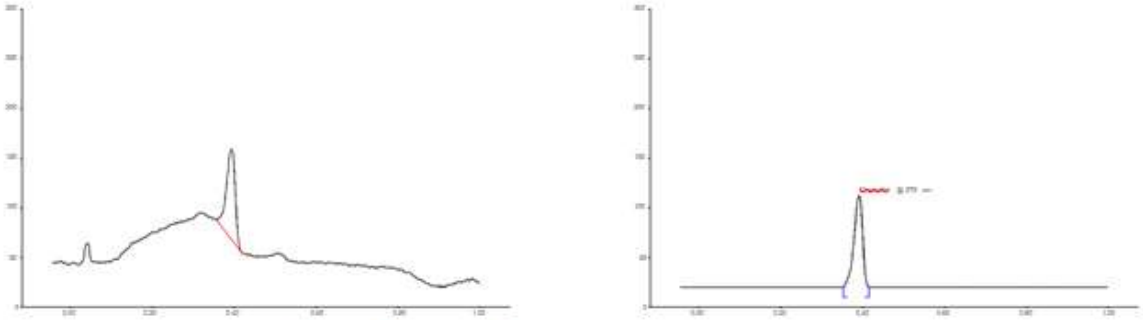


Figure 6: Spectra overlay of quercetin with corresponding peak in hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* aerial parts



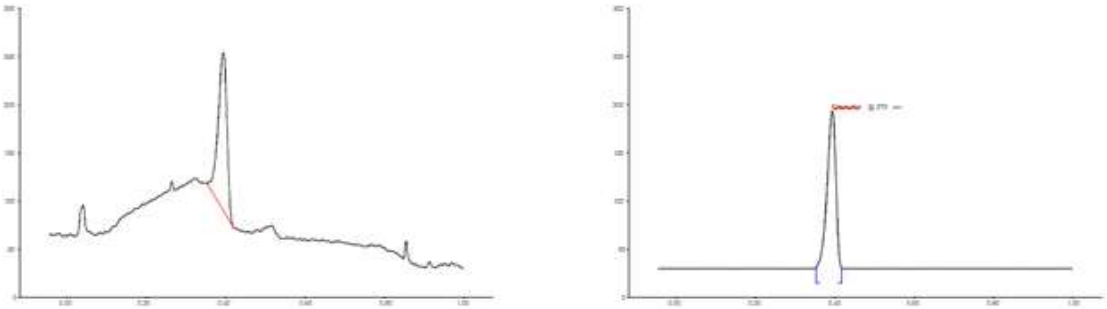
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.36	0.6	0.39	22.8	100.00	0.41	0.6	331.3	100.00	Quercetin

Track 2, ID: Standard2



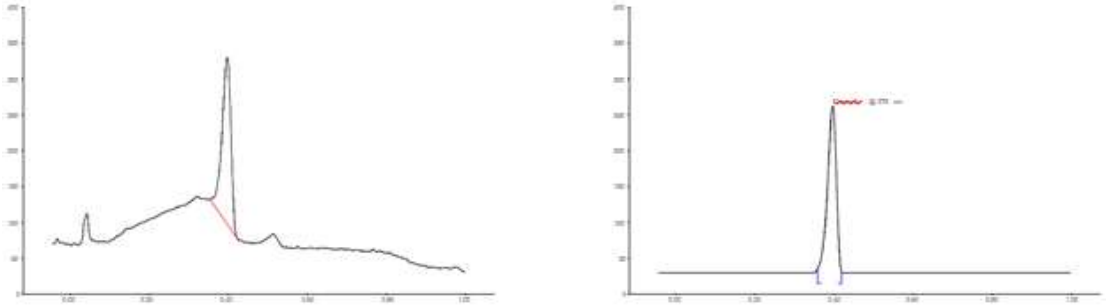
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	0.2	0.39	92.2	100.00	0.42	0.0	1515.5	100.00	Quercetin

Track 3, ID: Standard3



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	1.2	0.39	163.3	100.00	0.42	0.8	2797.3	100.00	Quercetin

Track 4, ID: Adenium obesum Extract



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.36	6.1	0.40	232.6	100.00	0.42	1.2	4042.0	100.00	Quercetin

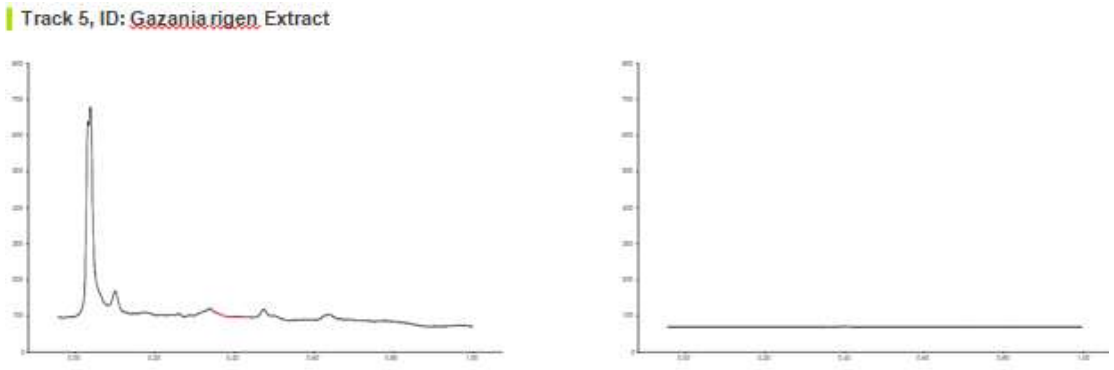


Figure 7: TLC Densitometric chromatogram of Standard (Track 1, 2, 3) and hydroalcoholic extracts of *Adenium obesum* (Track 4) and *Gazania rigens* (Track 5) aerial parts

Figure 8: Photographs of thin layer chromatography fingerprinting profile of hydroalcoholic extract of *Adenium obesum* and *Gazania rigens* aerial parts under 254 nm.

DISCUSSION

The hydroalcoholic extracts of *Adenium obesum* aerial parts had a greater concentration of total flavonoids compared to the hydroalcoholic extracts of *Gazania rigens* aerial parts. The hydroalcoholic extracts of *Gazania rigens* aerial parts had a greater concentration of total phenols compared to the hydroalcoholic extracts of *Adenium obesum* aerial parts. The chromatogram of hydroalcoholic extract of *Adenium obesum* and *Gazania rigens* aerial parts showed thirty seven and twenty six peaks, respectively, each representing a different phytoconstituents. According to our results (Table 4), small amount of quercetin detected in hydroalcoholic extracts of *Adenium obesum* and in *Gazania rigens* quercetin is not detected. Quercetin showed antidiabetic potential.[20] Presence of many phytoconstituents showed that both plants have anti-microbial, anti-inflammatory, anti-neoplastic, anti-diabetic, anti- oxidant activities. In *Adenium obesum,* phytoconstituents identified by GC-MS are Tetra ethyl silicate, silane, Pentadecane, Pthalic acid, Nonadecane etc have anti-microbial actitvity. Cyclopentene, Benzofuranone, Eicosane, etc have anti-inflammatory activity. 2 methyl hexacosane have anti diabetic activity.[21] 3,4-dihydroxyphenl- 5,7 dihydroxy 4H-1-benzopyran-4-one is quercetin which have anti diabetic activity.[22] In *Gazania rigens,* phytoconstituents identified by GC-MS are Estragole, Caryophyllene, Cryptomeridiol, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 etc have anti-microbial, anti-inflammatory activities.[23] 4-(3, 5-Di-tert-butyl-4-hydroxyphenyl)butyl acr has anti-oxidant activity. (3, 4-dihydroxyphenyl)prop-2-enoyl, oxy 1,4,5-trihydroxycyclohexane-1-carboxylic acid has glucose uptake activity.[24]

CONCLUSION

In the current study, the total phenolic, total flavonoid content was determined. *A. obesum* have high total flavonoid content while *G. rigens* have high total phenolic content. The phytochemical composition of hydroalcoholic extracts of *Adenium obesum* and *Gazania rigens* contain a diverse range of phytoconstituents including polyphenols, flavonoids, triterpenes, sterols, alkaloids and glycosides. Flavonoids and polyphenols have been extensively studied for their pharmacological properties, which include scavenging free radicals, reducing blood sugar levels, inhibiting microbial growth, and reducing inflammation. The pharmacological investigations conducted on extracts and isolated chemicals from these plants have demonstrated the potential of the plant, therefore providing justification for its traditional use as an antidiabetic medication, as anti-oxidants, as anti-microbial agents.

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