ESTABLISHMENT OF QUALITY PARAMETERS OF *Quisqualis indica* LEAVES THROUGH SOPHISTICATED ANALYTICAL TECHNIQUES

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ABSTRACT. *Quisqualis indica* (*Q. indica*; Rangoon creeper) is found in Asia and finds its place in Ayurvedic texts, ethno-medicine as well as modern research. Its leaves contain important constituents like rutin, quisqualis acid, trigonelline, L-proline and L-asparagine. Traditionally, the leaves are used as antipyretic, anti-flatulent, anti-inflammatory, anti-septic, and anti-diarrhoeal. Modern pharmacological research also supports these claims. However, this plant remains unexplored phytochemically, which restricts any means for standardization of its formulations. The present research focuses on analysis of leaves of *Q. indica* using sophisticated chromatographic and spectral techniques. Thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), gas chromatography-mass spectrometry (GC-MS) techniques were used. After several pilots, TLC analyses, an HPTLC fingerprint of methanolic extract of the leaves was performed using chloroform: methanol: ethyl acetate (7: 3: 3) solvent system, which showed 12 peaks at 254 nm and 9 peaks at 366 nm. GC-MS analysis of the methanolic extract detected 7 known phytochemicals, some of them having pharmacological importance. This research may serve the parameters for quality control of *Q. indica* leaves in herbal industries, in the detection of adulteration of its formulations as well as open new avenues for phytochemical research, including isolation of a marker compound.

KEY WORDS: *Quisqualis indica*, Adulteration, Fingerprinting, GC-MS, HPTLC, Standardization

INTRODUCTION

Quisqualis indica (*Q. indica*, Figure 1) is a ligneous vine that belongs to the family Combretaceae. In this plant, the leaves are opposite or elliptical. In India, it is grown as an ornamental plant, while it is distributed across the world in tropical countries, especially in China, the Philippines, Bangladesh, Myanmar, and Malaysia [1-3]. It is commonly known as Rangoon creeper or Chinese Honeysuckle (English). Local names of *Q. indica* include Madhumalati (Hindi), Modhumalati (Bengali), Parijat (Manipuri), Vilayati Chambeli (Marathi), Radha Manoharam (Telugu), Niyog-niyogan (Filipino), Quiscual (Spanish) and Shih-chun-tzu (Chinese) [4-5]. Each plant contains several phytochemicals in its various parts showing different pharmacological activities and toxicities, likewise *Q. indica* Linn. also shows many pharmacological activities due to the presence of medicinally active compounds.

 Q. indica leaves are rich in phytoconstituents including tannins, flavonoids, coumarins, steroids, carbohydrates, proteins, amino acids, saponins, and phenolic compounds. Its leaves contain major active phytoconstituents like stigmasterol, rutin, quisqualic acid, trigonelline, L-proline, and L-asparagine [4-6]. Flowers of *Q. indica* contain polyphenols, rutin, and pelargonidin-3-glucoside; seeds contain fixed oil (linoleic, oleic, stearic, and arachidic acids, sterols, alkaloids, and quisqualic acid) while fruits contain a sugary substance similar to levulose and an organic acid similar to cathartic acid [4].

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Figure 1. Flowers and leaves of *Quisqualis indica*.

The leaves are used for their antipyretic, anti-flatulent, anti-inflammatory, anti-septic, anti-staphylococcus, and anti-diarrheal properties. Additionally, the leaves have also shown antioxidant, antimicrobial, anthelmintic, analgesic, anticancer, antidiabetic, and immuno-modulatory activity with their use in boils and ulcers, gastric pain and dysentery [4-8].

Other parts of *Q. indica* also have significant ethnomedicinal uses. Fruits are tonic, and anthelmintic, used in nephritis and gargling, diarrhea, and as astringent. Seeds are used in diarrhea as an antiseptic, febrifuge for high fevers, vermifuge, and anthelmintic. Roots are used in rheumatism, diarrhea, and as anthelmintic [9-11].

This research study may aid in the identification and characterization of phytoconstituents by chromatographic fingerprints obtained using sophisticated HPTLC and GC-MS techniques. Using the reports, one can check adulteration and facilitate standardization of herbal formulations containing leaves of *Q. indica*. It will also promote further research studies and the isolation of phytochemicals for the betterment of the community.

EXPERIMENTAL

Chemicals and reagents

Methanol was purchased from the Rankeem Chem Trade Enterprise (purity >99%, analytical AR grade). Chloroform, ethyl acetate, ammonia and formic acid were obtained as gift sample from the Molychem Laboratory.

Collection and sampling

Five year old, mature fresh leaves of *Q. indica* were collected from the Medicinal Garden of RK University (Latitude 22.24006, Longitude 70.90098), Rajkot - India in the monsoon, August 2014,

and compared with standard literature for authentication. The leaves were ovate in shape, 5-13 cm × 2-5 cm in size, pale green in colour with coarse surface texture. A herbarium [SOP/COG/398/2014] was submitted to repository at School of Pharmacy, RK University, and certified by the Botanist from School of Science, RK University.

Extraction

After collection, the leaves were dried in a hot air oven at 50 ºC. Leaves of *Q. indica* were powdered and 25.0 g dry powder was extracted with 100 mL methanol for 24 hours by maceration at room temperature. The macerated solution was filtered and the filtrate was allowed to dry at 50 ºC. The obtained solid mass was stored for further experiments.

Pilot TLC and HPTLC studies

For the best resolution in HPTLC study, the mobile phase system was developed on TLC. Methanolic extract of leaves was used to develop TLC. Chloroform, methanol, ethyl acetate, ammonia and formic acid were used in the development of TLCs. After several trials in TLC studies, the best mobile phase system was identified. The proportions of the solvents were modified for several TLC plates to obtain precise and clear spots in fingerprinting. Chloroform: methanol: ethyl acetate (7: 3: 3) system gave best separation in TLC and forwarded for HPTLC analysis.

HPTLC analysis for fingerprinting of methanolic extract was carried out at the Department of Chemistry, Saurashtra University with chloroform: methanol: ethyl acetate (7: 3: 3) system. The HPTLC fingerprinting was obtained on HPTLC plates containing silica gel C_{60} F_{254} as stationary phase, manufactured by E. Merck KgaA. CAMAG Linomat 5 was used for sample application. Peak height and area were selected for evaluation, while the measurement was done using the principle of absorption. The sample was dissolved in Methanol. CAMAG TLC Scanner 3 was used for scanning the plates in daylight as well as at 254 nm and 366 nm.

GC-MS studies

Qualitative GC-MS analysis of the sample named Madhumalati, containing methanolic extract of *Q. indica* leaves was performed using GC-MS at the Department of Chemistry, School of Science, RK University – INDIA. Agilent 5977B MS occupied to 7820A GC was used to analyze. HP-5 capillary column (30 m × 0.32 mm; 0.25 μm film thickness) was used for GC-MS studies [12-16]. NIST library of the GC-MS was used to identify the detected compounds.

RESULTS AND DISCUSSION

Pilot TLC and HPTLC studies

The mobile phase chloroform: methanol: ethyl acetate (7: 3: 3) showed distinct spots in TLC. Hence, it was further used for HPTLC fingerprinting. HPTLC plates were scanned at 254 nm and 366 nm as shown in Figure 2 and their densitometric spectra are as shown in Figure 3.
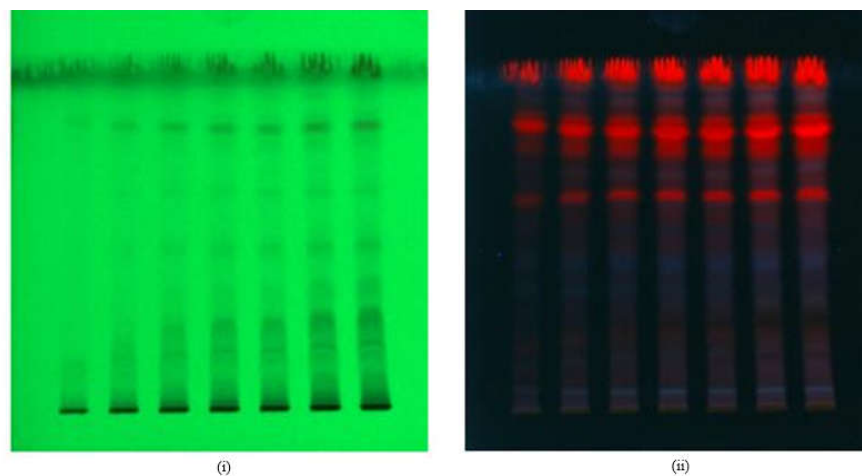
Figure 2. HPTLC plate scanned at (i) 254 nm and (ii) 366 nm.

Table 1. HPTLC - maximum R_f and Area under the curve at 254 nm.

Peak	Max R_f	AUC
1	0.17	2119.3
2	0.20	829.3
3	0.23	2792.2
4	0.33	561.7
5	0.40	216.5
6	0.45	1397.7
7	0.49	331.1
8	0.56	218.7
9	0.61	1933.1
10	0.66	432.7
11	0.72	934.6
12	0.76	3426.0

Table 2. HPTLC - maximum R_f and area under curve at 366 nm.

Peak	Max R_f	AUC
1	0.16	2792.4
2	0.18	3999.3
3	0.25	15701.1
4	0.34	5422.0
5	0.44	7090.6
6	0.59	4884.5
7	0.65	294.3
8	0.72	3508.8
9	0.76	13165.2

Methanolic extract of *Q. indica* leaves showed up at 12 spots at 254 nm (Table 1.) and 9 spots at 366 nm (Table 2.). HPTLC fingerprints may facilitate analysts to check adulteration by any means in herbal formulations to assure the quality by preventing foreign material as well as helping in standardization.
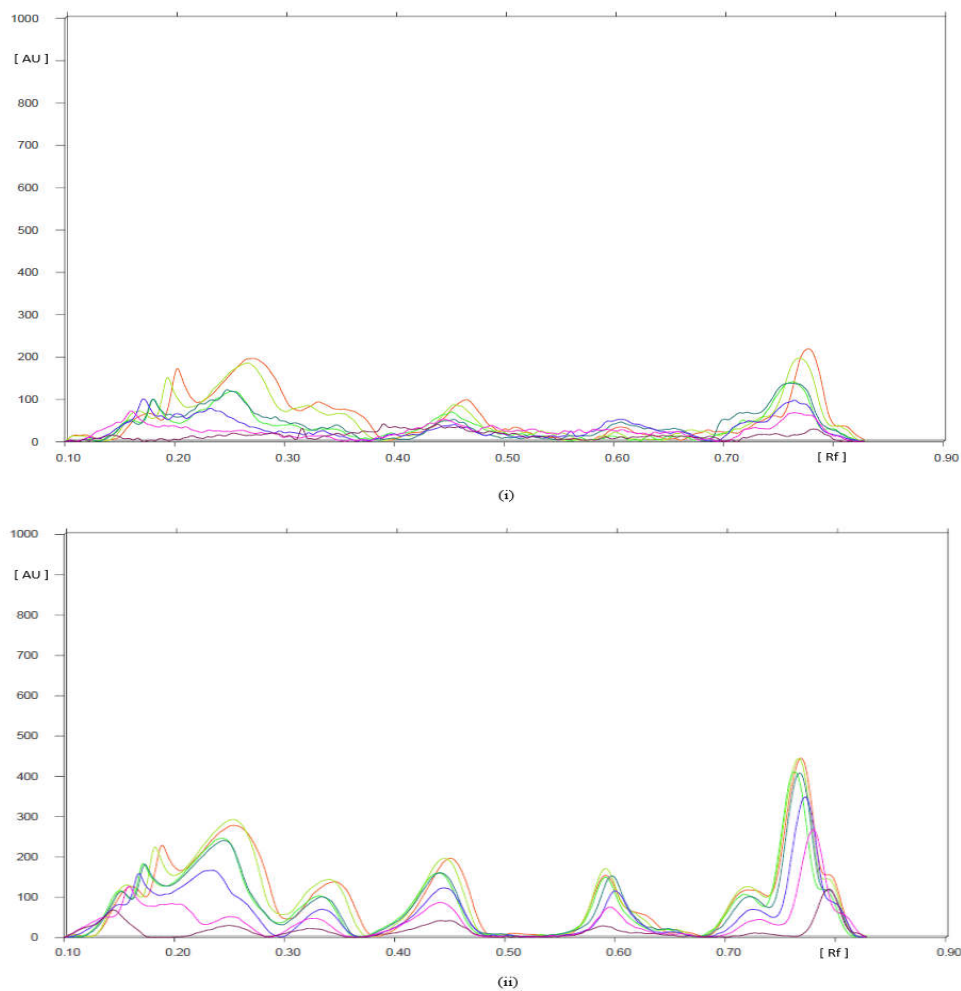
(i)

(ii)

Figure 3. 2D Densitometric chromatogram of methanolic extract of *Q. indica* leaves (i) 254 nm and (ii) 366 nm.

GC-MS studies

From GC-MS analysis of the methanolic extract of *Q. indica* leaves, 7 phytochemicals as shown in Table 3 were identified using NIST library. Chromatogram of the GC analysis is shown in Figure 4, while spectra from the MS analysis is shown in Figure 5.
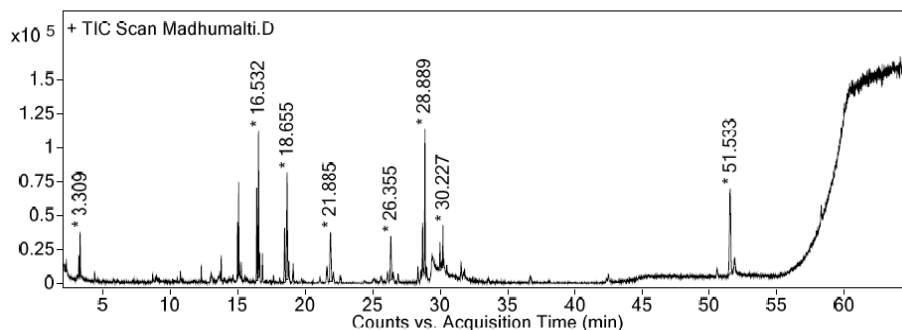
Figure 4. Gas chromatogram of methanolic extract of *Q. indica* leaves.

Table 3. Phytochemicals identified from methanolic extract of *Q. indica* leaves.

| Peak	RT	Name of compound	Mol. formula	Mol. wt.	Peak area %
1	14.971	1-Undecanol	$C_{11}H_{24}O$	172.31	3.2
2	15.069	1-Fluoro-dodecane	$C_{12}H_{25}F$	188.32	4.52
3	16.532	Decylundecyl ester carbonic acid	$C_{22}H_{44}O_3$	356.6	11.48
4	18.477	1-Dodecanol	$C_{12}H_{26}O$	186.33	6.24
5	21.885	Decylheptyl ether	$C_{17}H_{36}O$	270.5	8.83
6	28.889	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	13.12
7	51.533	3,5-Dedihydro–stigmastan-6,22-diene	$C_{29}H_{46}$	394.676	17

Compound 1. 1-Undecanol, Mol. formula: $C_{11}H_{24}O$, Mol. wt.: 172.31, RT: 14.971. GC-MS fragment: The peak at 14.971 minutes had a mass [M$^+$] 172.31. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 56.1, 57.1, 67, 69.1, 70.1, 71.1, 83.1, 97.1, 111.1.

Compound 2. 1-Fluoro-dodecane, Mol. formula: $C_{12}H_{25}F$, Mol. wt.: 188.32, RT: 15.069. GC-MS fragment: The peak at 15.069 minutes had a mass [M$^+$] 188.32. The daughter ion spectra of the compound revealed the characteristic fragments m/z 54, 55.1, 56.1, 57.1, 69.1, 70.1, 71.1, 83, 85.1, 99.

Compound 3. Decylundecyl ester carbonic acid, Mol. formula: $C_{22}H_{44}O_3$, Mol. wt.: 356.6, RT: 16.532. GC-MS fragment: The peak at 16.532 minutes had a mass [M$^+$] 356.6. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 56.1, 57.1, 69.1, 70.1, 71.1, 83.1, 85.1, 97.1, 99.1.

Compound 4. 1-Dodecanol, Mol. formula: $C_{12}H_{26}O_3$, Mol. wt.: 186.33, RT: 18.477. GC-MS fragment: The peak at 18.477 minutes had a mass [M$^+$] 186.33. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 56, 57.1, 67, 69.1, 70.1, 83.1, 83.1, 97.1, 111.1.

Compound 5. Decylheptyl ether, Mol. formula: $C_{17}H_{36}O$, Mol. wt.: 270.5, RT: 21.885. GC- MS fragment: The peak at 21.885 minutes had a mass [M$^+$] 270.5. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 56.1, 57.1, 69.1, 70.1, 71.1, 71.1, 83.1, 85.1, 97.1.
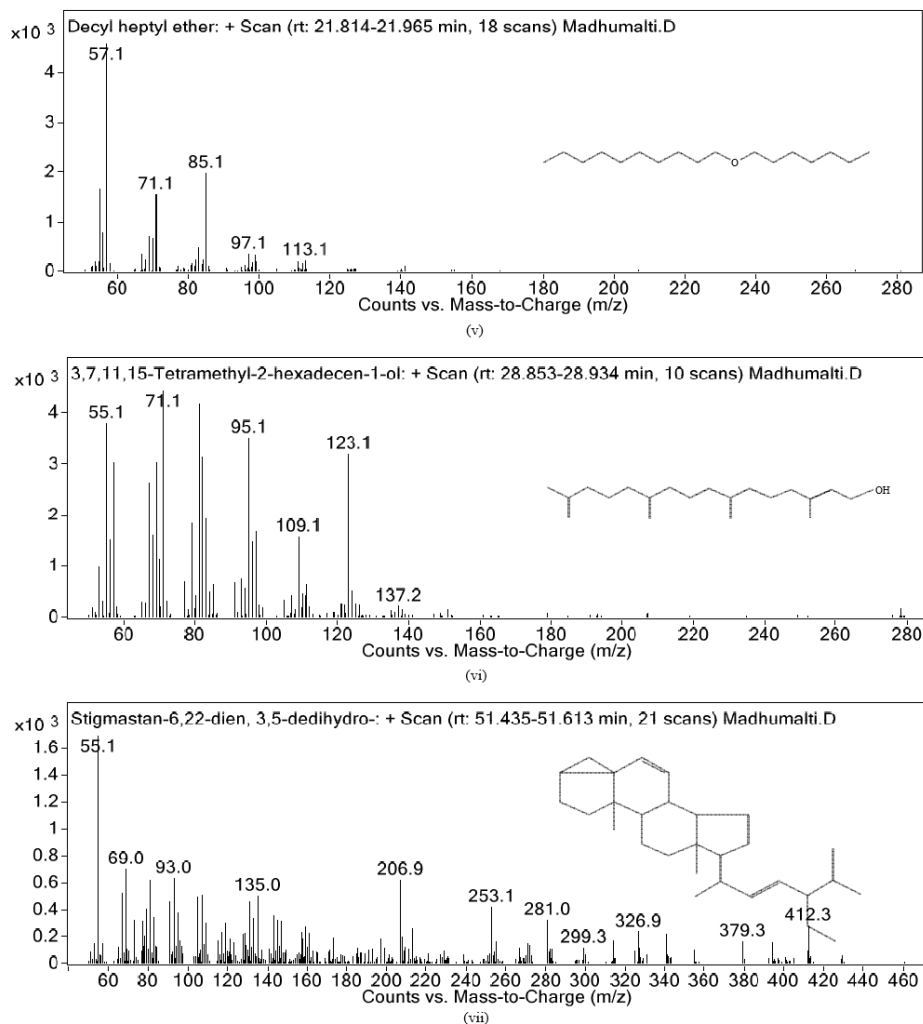
(i)

(ii)

(iii)

(iv)
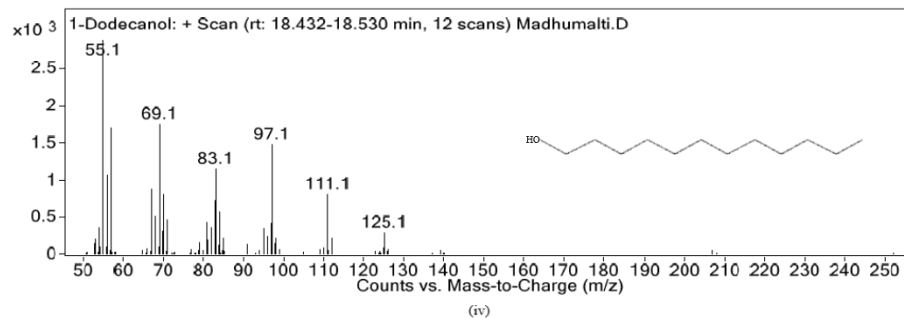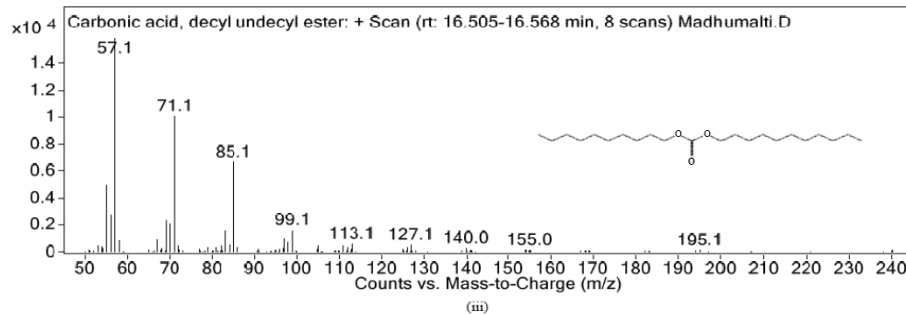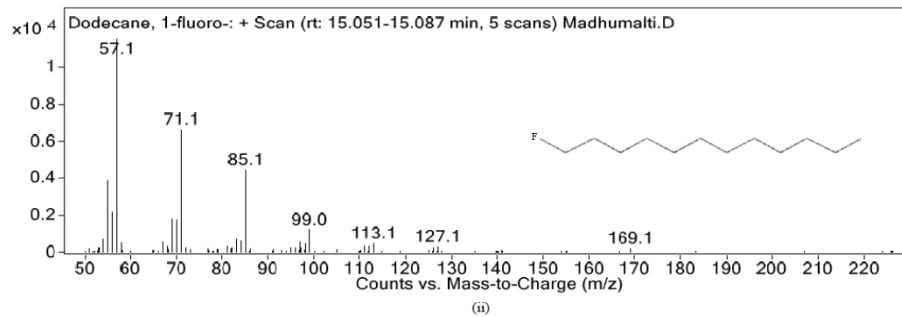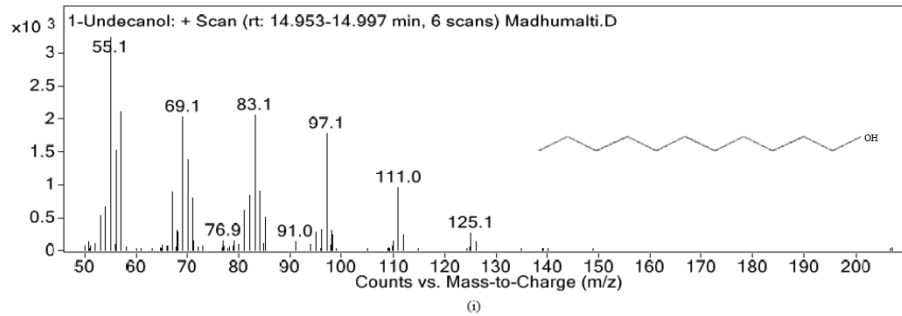
Figure 5. Mass spectra of compounds (i) 1-undecanol, (ii) 1-fluoro-dodecane, (iii) decylundecyl
ester carbonic acid, (iv) 1-dodecanol, (v) decylheptyl ether, (vi) 3,7,11,15-tetramethyl-
2-hexadecen-1-ol and (vii) 3,5-dedihydro–stigmastan-6,22-diene.

Compound 6. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Mol. formula: $C_{20}H_{40}O$, Mol. wt.: 296.5, RT: 28.889. GC-MS fragment: The peak at 28.889 minutes had a mass $[M^+]$ 296.5. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 57.1, 67.1, 69.1, 71.1, 81.1, 82.1, 83.1, 95.1, 123.1.

Compound 7. 3,5-Dedihydro–stigmastan-6,22-diene, Mol. formula: $C_{29}H_{46}$, Mol. wt.: 394.676, RT: 51.533. GC-MS fragment: The peak at 51.533 minutes had a mass $[M^+]$ 394.676. The daughter ion spectra of the compound revealed the characteristic fragments m/z 55.1, 67, 69, 81.1, 93, 105.1, 107, 131, 135, 206.9.

The phytochemicals detected from the GC-MS analysis of the methanolic extract of *Q. indica* leaves were reported with significant biological activities. Decylundecyl ester carbonic acid is used as an acidifier as well as to inhibit the production of uric acid. 3,7,11,15-tetramethyl-2-hexadecen-1-ol is useful for providing oligosaccharides to the body. However, others did not possess known pharmacological activities.

It is significant to note that Agarwal *et al.* [17] reported 15, 12, and 18 compounds qualitatively via GC-MS analysis, in methanol, ethyl acetate, and hexane extracts respectively [17]. In addition to that, Sutar *et al.* [18] investigated the phytochemical and biological activities of *Q. indica leaves* and evaluation of secondary metabolites and characterization of isolated compounds was done by TLC, GC-MS analysis, NMR, and FTIR [18]. Potphode *et al.* had performed bioethanol production of *Q. indica* leaves by GC-MS analysis [19].

CONCLUSION

This exclusive research work may open up further ideas to facilitate the standardization of herbal formulations containing *Q. indica* leaves. The obtained chromatographic fingerprints may aid to check adulteration and in the quality control of herbal formulations. This research study can also be used in further research for the isolation of compounds or as a marker for other processes.

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