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HPTLC Analysis of hydro alcoholic extracts of *Clerodendrum viscosum* V. leaves and *Macrotyloma uniflorum* L. seeds

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ABSTRACT

The objective of this study was to perform the HPTLC analysis of hydro alcoholic extracts of *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L. seeds. HPTLC is a powerful analytical tool in the identification of chemical constituents in plant extracts. Leaves of *Clerodendron viscosum* V. and seeds of *Macrotyloma uniflorum* L. are having numerous traditional usages ranging from wound healing to the treatment of tumors. Hydro alcoholic extracts of *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L. seeds were obtained using cold maceration. The obtained extracts were subjected to HPTLC analysis by using the instrument "CAMAG Linomat 5". Quercetin, Rutin and Gallic acid were used as standards; Toluene: Ethyl Acetate: Formic Acid: Methanol (3:6:1.6:0.4) solution was used as a mobile phase. 10 µL of plant extracts and 5 µL of standard solutions were applied during the analysis. Both the plant extracts have shown the presence of Quercetin as one of the major constituent which is having numerous therapeutic properties. Extract of *Macrotyloma uniflorum* L. seeds has shown the presence of Quercetin with 0.91 Rf, 19.93 % Area, and 3655.7 Area; whereas the *Clerodendron viscosum* V. leaves extract has shown the presence of Quercetin with 0.90 Rf, 19.17 % Area, and 1685.9 Area.

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INTRODUCTION

High-performance thin layer chromatography (HPTLC) is the advancement of thin layer chromatography with sophisticated features like automation, scanning, full optimization, selective detection principle, minimum sample preparation, and hyphenation etc. This technique will analyze the sample qualitatively as well as quantitatively and gives the results in an image format. HPTLC – finger printing analysis is a powerful tool to identify the chemical constituents present in the given plant extract (Attimarad *et al*. 2011).

Clerodendrum viscosum V. (Synonyms: *Clerodendrum infortunatum* Linn. and *Clerodendrum calycinum* Turcz.*)* belongs to Family: Verbenaceae, Kingdom: Plantae, Genus: Clerodendron. It is called as Hill glory bower in English, Bhat in Hindi, Ghentu in Bengali and Bhania in Oriya. It is a terrestrial shrub, 2-4 feet in height, widely distributed in various parts of India, Ceylon, Malaya and Bangladesh (Kirtikar & Basu 2001). Traditionally, the plant was used in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation, epilepsy, postnatal care, to dress fresh wounds, in tumors, cirrhosis, jaundice, in scorpion-sting, and snake-bite (Gupta & Sharma 2008; Modi, Khadabadi & Deore 2010). Authors have reported that *Clerodendrum viscosum* V. possess Antimicrobial

(Waliullah *et al.* 2014), Anthelmintic (Modi, Khadabadi & Deore 2010), Analgesic & Anticonvulsant (Pal, Sannigrahi, & Mazumder 2009), Antihyperglycemic (Baid 2013; Das *et al.* 2011) Anti-inflammatory (Khatri 2005; Das *et al.* 2010), Anticancer (Sannigrahi *et al.* 2012), Hepatoprotective (Sannigrahi *et al.* 2009), Wound healing (Kuluvar *et al.* 2009), Antifeedant (Abbaszadeh, Srivastava & Walia 2014) and Nootropic potential (Gupta & Singh 2012) activities. *Clerodendrum viscosum* V. leaves contains saponins, alkyl sterols, some enzymes and fixed oil consists of glycerides of lenoleic, oleic, stearic and lignoceric acid (Prajapati *et al.* 2001; Kapoor 2001).

Macrotyloma uniflorum Lam. (Verdc.) (Synonyms: *Dolichos biflorus Auct.* and *Dolichos uniflorus Lam.)* belongs to Family: Fabacae, Subfamily: Faboideae, Tribe: Phaseolae, Sub tribe: Phaseolinae, Kingdom: Plantae, Genus: Macrotyloma. It is called as Kurtikalai in Bengali; Horse gram, horse grain, kulthi bean, madras bean, madras gram, poor man's pulse in English; Muthira in Malayalam; Kulattha in Sanskrit; Kollu in Tamil and Ulavalu in Telugu. It is widely distributed in various parts of Africa, Australia, Bhutan, India, Indonesia, Myanmar, Nepal, Pakistan, Philippine and Sri-Lanka (Blumenthal & Staples 1993; Nasir 1981). In the traditional system of medicine, the seeds of *Macrotyloma uniflorum* Lam. (Verdc.) were used for heart diseases, asthma, bronchitis, leucoderma, urinary discharges, inflamed joints, fever, sinus wounds and localized abdominal tumors (Muthu *et al.* 2006; Kaswar *et al.* 2009). The seeds of the plant was reported to have various pharmacological activities such as Anticholelithiatic (Bigoniya, Bais, & Sirohi 2014), Antihistaminic (Suralkar, & Kasture 2013), Anti-Peptic Ulcer (Panda, & Suresh 2015), Antioxidant (Singh *et al.* 2012; Ravishankar, & Priya 2012;

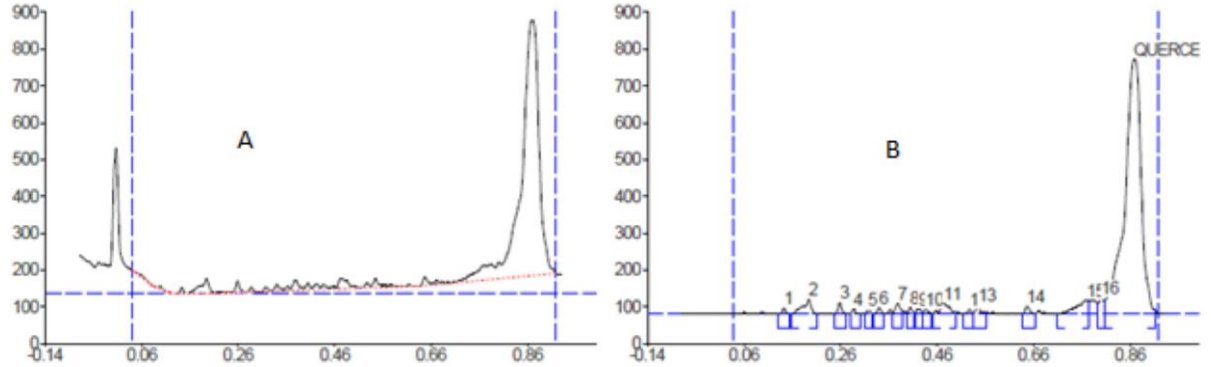

Figure 1: A: Peak Baseline of Quercetin B: Peak Densitogram of Quercetin at 254 nm

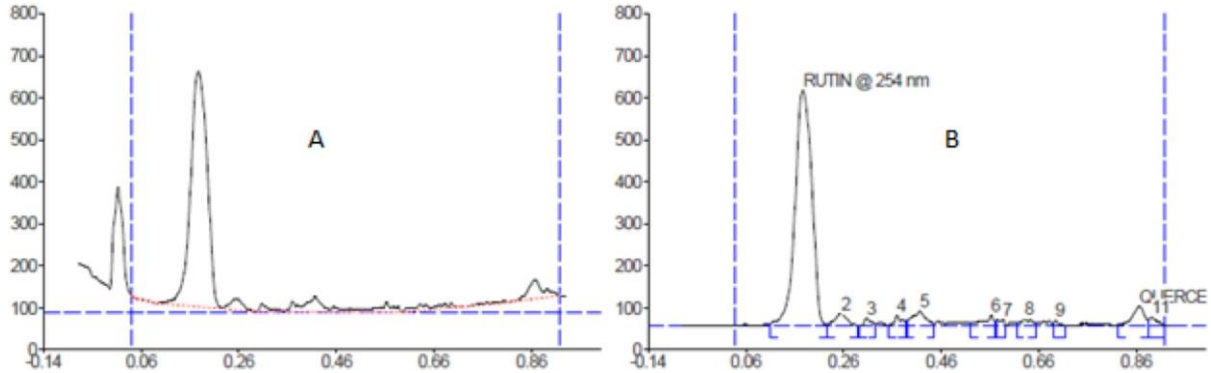

Figure 2: A: Peak Baseline of Rutin B: Peak Densitogram of Rutin at 254 nm

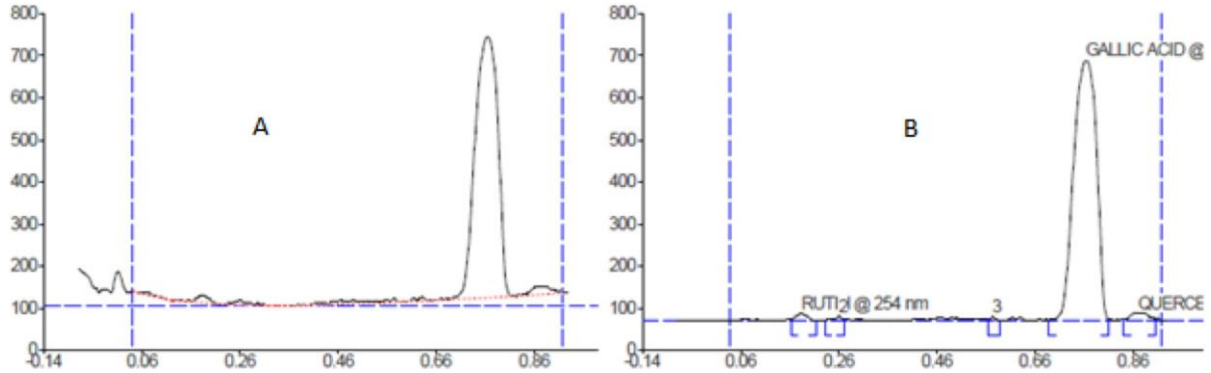

Figure 3: A: Peak Baseline of Gallic Acid B: Peak Densitogram of Gallic Acid at 254 nm

Marimuthu, & Krisnamoorthi 2013), Antiobesity (Sengupta *et al.* 2012), Anti-Uroliathiatic against Calcium Oxalate Crystals (Das *et al.,* 2005; Chaitanya *et al.* 2010; Atodariya *et al.* 2013; Bijarnia *et al.* 2009), Anti-Uroliathiatic against Calcium Phosphate Crystals (Kieley, Dwivedi, & Monga 2008),

Anti-Uroliathiatic against Uric Acid Crystals (Ahmad *et al.* 1992), Antimicrobial, & Anticancer (Chakraborty, & Abraham 2016), Hemolytic (Kaswar *et al.* 2009), Hepatoprotective (Parmar 2012), Larvicidal (Gupta *et al.* 2011), Proteinase Inhibition (Ramasarma, Rao, & Rao 1995; Sreerama *et al.* 1997), Nephrotoxicity Management (Saha, & Verma 2012), Antidiabetic and Antihypertensive Activity through ACE-1 Inhibition (Wagner *et al.* 1991; Chen *et al.* 1992). Seeds contains Anthocyanins, Flavonoids, and Phenolic acids (Sreerama, Sashikala, & Pratape 2010; Kawsar *et al.* 2008). The seed also contains α – Amylase (Garg, & Dobriyal 2011), β-N-Acetylucosaminidase, α -and β Glucosidase (Meyer, & Bourrillon 1973), Urease (Natarajan 1995), Haemagglutinins, Tannins (Bhartiya, Aditya, & Kant 2015)and Phytic acid (Sreerama *et al.* 2012; Sreerama, Sashikala, & Pratape 2010).

MATERIALS AND METHODS

Collection and Authentication of the Plant

The leaves of *Clerodendrum viscosum* V. & seeds of *Macrotyloma uniflorum* L. were collected from Erattayal, Palakkad dist., Kerala, India. Dr. Harsha Hegde, Scientist 'B' has authenticated these leaves and seeds at Regional Medical Research Centre (RMRC), Indian Council of Medical Research (ICMR), Belgaum. A voucher specimen of *Clerodendrum viscosum* V. leaves & *Macrotyloma uniflorum* L. seeds have been deposited in the herbarium of RMRC with accession number RMRC-1123 and RMRC-1167 respectively.

Preparation of the Extracts

The collected leaves and seeds were shade dried (28±3°C) for 7 days and dried in hot air oven (50±3°C). The dried leaves and seeds were ground and sieved to get fine powder and subjected to cold maceration with water and hydro alcohol (1:1 Water: Ethanol) separately in a shaker system at room temperature to obtain aqueous and Hydro alcoholic extracts respectively. Each extracts were filtered, and the filtrate was subjected to evaporation under reduced pressure to obtain dry extract.

Standard Chemicals

Quercetin, Rutin and Gallic Acid were obtained from Sigma chemicals, USA.

Preparation of Standard solutions

Standard solution was prepared by dissolving 1mg of standard i.e. Quercetin, Rutin and Gallic Acid was separately mixed with 1ml Chloroform and used for the analysis. Peak baseline and peak densitogram of Quercetin, Rutin and Gallic Acid were represented in Fig. 1., Fig.2. and Fig.3. respectively.

Preparation of Test solutions

Each of the plant extracts were centrifuged at 3000rpm for 5min. The resultant solution was used as test solution for HPTLC analysis.

Sample application

10 µl of test solution and 5 µl of standard solutions were loaded as 6mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe. "CAMAG LINOMAT 5" instrument was used for the HPTLC analysis.

Mobile Phase

Toluene, Ethyl Acetate, Formic Acid and Methanol were mixed in the ratio of 3:6:1.6:0.4 and the resultant solution was used as a mobile phase.

Spot development

The sample loaded plate was kept in 10x10cm Twin Trough Chamber (after saturated with Solvent vapor) with the mobile phase up to 80mm.

Scanning

The developed plate was dried by oven (at 60°C) to evaporate solvents from the plate. The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 254nm using D2 & W lamp. The Peak table, Peak Baseline, and Peak display were noted. The software used was winCATS 1.3.4 version.

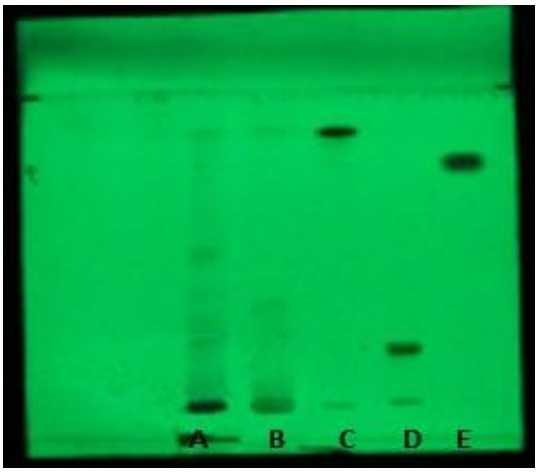

Figure 4: Chromatogram of A: hydro alcoholic extract of *Macrotyloma uniflorum* L. seeds B: hydro alcoholic extract of *Clerodendrum viscosum V. leaves* C: Quercetin D: Rutin E: Gallic Acid at 254 nm

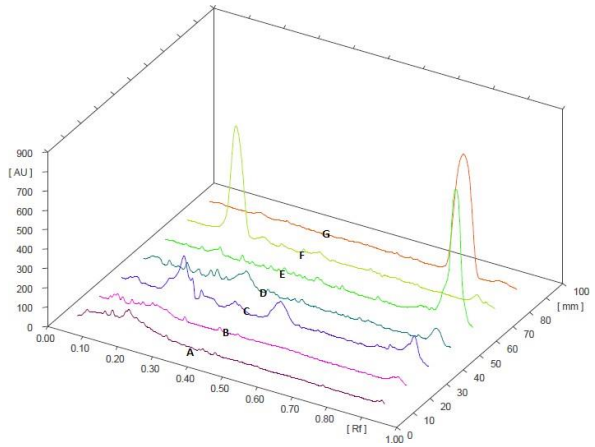

Figure 5: 3D Chromatogram of all tracks at 254 nm A: Test 1 B: Test 2 C: hydro alcoholic extract of *Macrotyloma uniflorum* L. seeds D: hydro alcoholic extract of *Clerodendrum viscosum V. leaves* E: Quercetin standard F: Rutin standard G: Gallic Acid standard

RESULTS AND DISCUSSION

The hydro alcoholic extract of *Macrotyloma uniflorum* L. seed has showed 9 different compounds. Out of these 9 compounds, eight compounds were unknown; whereas one compound was found to be Quercetin with 0.91 Rf value (matched with standard quercetin Rf value 0.91), 19.93 % area and 3655.7 Area. Among eight unknown compounds, the constituents representing 2nd and 6th peaks were available more than Quercetin in the extract which are showing % Area 35.03 and 21.15 respectively. Chromatogram of both plant extracts and three standard compounds were presented in Fig.4. 3D Chromatogram of all the tracks (Test 1, Test 2, hydro alcoholic extract of *Macrotyloma uniflorum* L. seeds, hydro alcoholic extract of *Clerodendrum viscosum V. leaves,* Quercetin standard, Rutin standard and Gallic Acid standard) has been depicted as Fig.5. The peak table, peak baseline and peak densitogram of *Macrotyloma uniflorum* L. seed extract was presented as Table.1. and Fig.6. respectively.

A total of 18 compounds were identified in the HPTLC analysis of hydro alcoholic extract of *Clerodendrum viscosum* V. leaves. Among these 18 compounds, one compound was found to be Quercetin with 0.90 Rf value (near to standard quercetin Rf value 0.91), 19.17 % area and 1685.9 area. The compound representing the 9th peak is highest ratio of availability in the extract which is showing 0.36 Rf value, 32.23 % area and 2834.2 area. The peak table, peak baseline and peak densitogram of *Clerodendrum viscosum* V. leaves extract was presented as Table.2. and Fig.7. respectively.

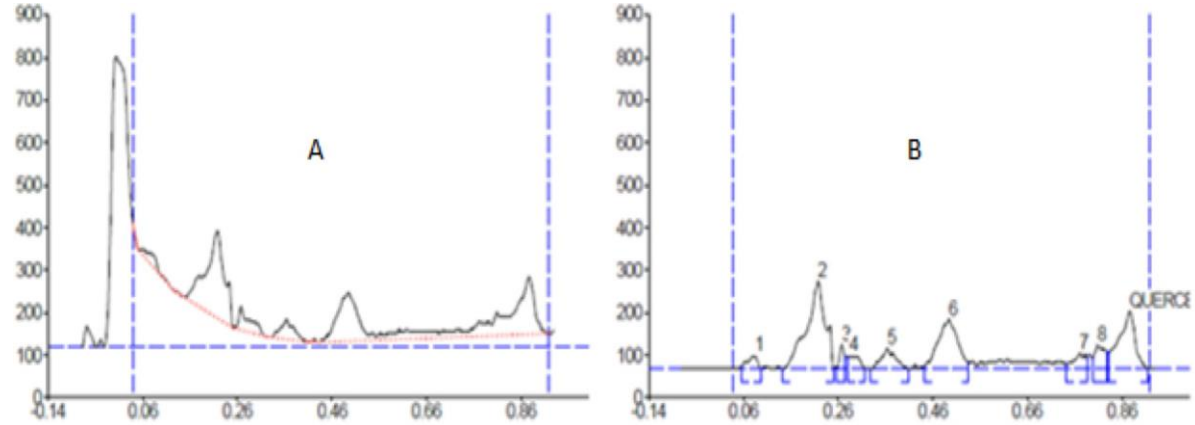

Figure 6: A: Peak Baseline of hydro alcoholic extract of Macrotyloma uniflorum L. seeds (B: Peak Densitogram of hydro alcoholic extract of *Macrotyloma uniflorum* L. seeds at 254 nm)

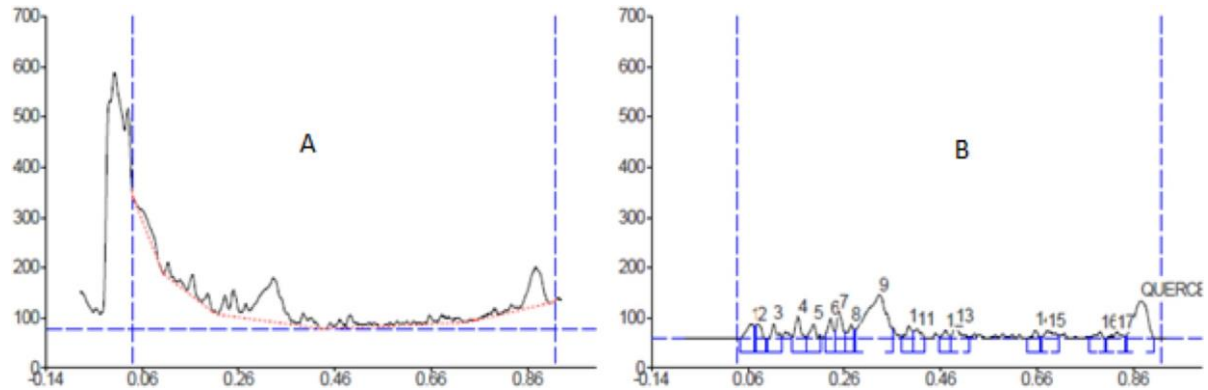

Figure 7: A: Peak Baseline of hydro alcoholic extract of *Clerodendrum viscosum V. leaves* B: Peak Densitogram of hydro alcoholic extract of *Clerodendrum viscosum V. leaves* at 254 nm

Table 1: Peak table with Rf values, height and area of Quercetin and unknown compounds in hydro alcoholic extract of Macrotyloma uniflorum L. seeds

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.05	6.5	0.08	29.1	4.13	0.09	0.9	516.6	2.82	Unknown *
2	0.14	0.1	0.22	204	28.98	0.25	1.5	6424.8	35.03	Unknown *
3	0.25	2.9	0.27	54.5	7.74	0.27	27.7	428.2	2.33	Unknown *
4	0.28	29.3	0.28	29.8	4.23	0.32	0.4	561.7	3.06	Unknown *
5	0.33	0.0	0.36	47.8	6.79	0.41	0.1	1127.0	6.14	Unknown *
6	0.44	5.6	0.49	114.9	16.32	0.54	13.4	3879.5	21.15	Unknown *
7	0.74	11.5	0.77	35	4.97	0.79	29.5	746.0	4.07	Unknown *
8	0.80	26.4	0.81	54.5	7.74	0.83	41.7	1002.9	5.47	Unknown *
9	0.83	41.6	0.88	134.4	19.09	0.91	0.2	3655.7	19.93	Quercetin

Table 2: Peak table with Rf values, height and area of Quercetin and unknown compounds in hydro alcoholic extract of Clerodendrum viscosum V. leaves

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.04	0.5	0.07	28.1	4.78	0.07	24.6	384.0	4.37	Unknown *
2	0.07	25.3	0.08	28.3	4.82	0.09	0.0	273.9	3.11	Unknown *
3	0.10	0.0	0.11	29.8	5.07	0.13	3.6	235.8	2.68	Unknown *
4	0.15	3.5	0.16	43.2	7.35	0.18	2.5	377.5	4.29	Unknown *
5	0.18	3.2	0.20	29.3	4.98	0.21	5.8	264.9	3.01	Unknown *
6	0.22	6.3	0.23	40.1	6.83	0.24	14.1	346.1	3.94	Unknown *
7	0.24	14.9	0.25	53.9	9.17	0.26	11.9	497.5	5.66	Unknown *
8	0.26	12.1	0.27	27.9	4.74	0.28	16.5	246.3	2.8	Unknown *
9	0.28	18.1	0.33	87.2	14.84	0.36	21.0	2834.2	32.23	Unknown *
10	0.38	4.4	0.39	25.8	4.38	0.40	14.3	236.4	2.69	Unknown *
11	0.40	14.4	0.41	18.8	3.19	0.43	0.0	212.3	2.41	Unknown *
12	0.46	3.5	0.47	15.0	2.56	0.48	4.5	148.2	1.69	Unknown *
13	0.48	4.8	0.49	25.2	4.29	0.52	3.7	301.9	3.43	Unknown *
14	0.64	0.9	0.66	17.4	2.97	0.67	4.1	146.8	1.67	Unknown *
15	0.67	4.1	0.68	15.9	2.70	0.70	5.4	267.1	3.04	Unknown *
16	0.77	3.2	0.79	12.3	2.09	0.80	1.6	152.2	1.73	Unknown *
17	0.80	1.9	0.83	14.1	2.40	0.84	5.0	181.9	2.07	Unknown *
18	0.84	5.4	0.88	75.5	12.84	0.90	0.7	1685.9	19.17	Quercetin

CONCLUSION

Hydro alcoholic extracts of *Clerodendrum viscosum* V. leaves & *Macrotyloma uniflorum* L. seeds were shown the presence of Quercetin as one of the major constituent. Quercetin is having numerous proven therapeutic properties; which may be attributed to the therapeutic activities of both the plant extracts. Since, there are high numbers of unknown compounds are present in the extract; further research work needs to be carried out to identify these unknown compounds.

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