International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 04-08-2021, Accepted: 18-08-2021, Published: 08-09-2021
Volume 6, Issue 5, 2021, Page No. 19-26

HPLC Identification of active flavonoid and phenolic compounds from leaf extracts of *Diospyros chloroxylon* Roxb.

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Abstract
Diospyros is the most versatile genus belonging to family Ebanceae. It comprises more than 500 species distributed in tropical and warm temperate regions of the world. These species have been used in the traditional medical systems of several countries. Therefore, the *Diospyros* species drawn the attention of research investigators to analyze their phytochemical profile and biological activities. The present study relates to phytochemical screening of *Diospyros chloroxylon* leaf extracts through HPLC technique. Twelve phenolic and flavonoid compounds (*viz*., (1) Apigenin-6, 8-di-C-glucopyranoside (2) Kaempferol- 3, 7-di-O-rhamnosid (3) Kaempferol-7-O-rhamnoside (4) Isorhamnetin (5) Isorhamnetin-3-O-dihexoside (6) Scutellarein7-O-β-D-glucopyranoside (7) gallic acid (8) Quercetin (9) Kaempferol (10) Rutin (11) (−) epigallocatechine and (12) Epicatechin gallate) were identified in the aqueous and methanolic leaf extracts of *Diospyros chloroxylon*. The above compounds confirm the medicinal potential of *Diospyros chloroxylon* and emphasizes the need of further pharmacological studies in this species.

Keywords: phenoloics, flavonoids, HPLC protocols, medicinal plants, pharmacological importance

Introduction
Plants contain the bioactive compounds useful in many biological activities (Rahman *et al* 2018) [16]. Flavonoids are an important antioxidant agents. Phenolic compounds are the most commonly occurring group of phytochemicals and play a potential protective role against various kinds of oxidative diseases *viz*., antimutagenicity, anti-bacterial action, antiviral activity, anti-inflammatory and apoptotic actions (Glucin *et al* 2010 & 2011) [5, 6]. Natural flavonoids and phenols exhibited many protective therapeutic potential against dreadful diseases. Flavonoids present in fruits and leafy vegetables are responsible for useful health benefits through radical scavenging and chelating activity. Several studies have suggested that rutin, kaempferol, quercetin, apigenin etc., are well-known for their anti-inflammatory, anti-allergic, anti-thrombotic, hepatoprotective, anti-spasmodic and anticancer properties (Mahesh Kumar & Kerti 2012) [13]. The chromatographic techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), nuclear magnetic resonance (NMR) and mass spectrometry have led to the identification of multitude of bioactive ingredients from many plant species (Sasidharan *et al* 2011, Katiyar *et al* 2012) [19, 8]. In recent times 'High performance liquid Chromatography (HPLC)' gained prominence for the analysis of plant extracts. This led to increasing interest in the isolation, identification and study of the biological activities of natural compounds. HPLC allows a high resolution rapid and reproducible determination of trace amounts of these compounds. The different naphthoquinens, anthroquinones, terpenoids, phenolic acids and flavonoids have been identified from *Diospyros* species (Rauf *et al* 2017) [17]. Because of the antioxidant, hepatoprotective, anti-inflammatory, analgesic, antipyretic, antihypertensive, antidiabetice, neuroprotective, antimicrobial, antiprotozal, fungicidal, antihelmintic, insecticidal, mollucidal, cytotoxicity, anti-tumor, multi drug resistance (MDR) reversal activities reported in *Diospyros* genus, the species have ever been considered as the most important medicinal species. Hence, the present study is aimed at the identification of some biological compounds (i.e, phenolics and flavonoids) from methanolic and aqueous leaf extracts.

Material and methods
HPLC instrument
Agilent 1100 series HPLC with Quaternary G1311 A pump, COLCOM G1316A thermostat column temperature control, Thermostatic auto sampler G 1329A with sample volume of 0. 1 – 1500 µL and variable programmable UV detector G 1314 A. The instrument was operated and integrated with Agilent chem. station LC software. High Performance Liquid Chromatographic (HPLC) protocols of Zang *et al* (2010) [23], Sait *et al* (2015) [18] and Hilal Bardakci *et al* (2018) [7] were used to identify different flavonoids in the aqueous leaf extracts of *Diospyros chloroxylon*. The HPLC protocols standardized by Adriana Skendi *et al* (2017) [2], Gini & Jothi (2018) [4] and Nadia Zeghad *et al* (2019) [14] were used for the identification of phenolic compounds in the methanolic leaf extracts of *Diospyros chloroxylon*.

Identification of flavonoid compounds through HPLC
Chemicals and Solvents
HPLC Grade Methanol is purchased from Thermo Fisher Scientific India private limited, Mumbai.
HPLC Grade Acetonitrile is purchased from Merck chemicals private limited, Mumbai.
HPLC Grade Water is purchased from Thermo Fisher Scientific India private limited, Mumbai other chemicals

used were of Laboratory reagent grade and were purchased from Merck chemicals, Mumbai.

Test method 1 (Zang *et al* 2010) [23].
Column: ZORBAX ODS C18 column (4.6 × 12.5 mm id, 5 µm).

Mobile phase: solvent A (0.1% formic acid aqueous, V/V, pH 2.6) and solvent B (acetonitrile).

Gradient program
0-10 min, linear gradient 10.0%-20.0% B; 10-20 min, isocratic 20.0% B 20-25 min, linear gradient 20.0%-30.0% B; 25-35 min, linear gradient 30.0%-33.0% B
35-40 min, linear gradient 33.0%-40.0% B; 40-60 min, linear gradient 40.0%-60.0% B

Mobile phase flow rate: 0.8 mL·min-1;
Sample volume: 20 µL; **Colum temperature:** 30°C; **Detector wavelength:** 254 nm

Test method 2 (Sait *et al* 2015**)** [18]**.**
Column: Ascentis C18 column (250 mm×4.6 mm I.D., 5 µm).
Mobile phase: (A) 0.1M HCOOH in H_2O and (B) ACN.

Gradient program
0-15 min from 10% to 20% B; 15-35 min from 20% to 30% B; 35-45 min from 30% to 50% B

Mobile phase flow rate: 1.0 mL·min-1
Sample volume: 20 µL;
Colum temperature: 30°C;
Detector wavelength: 352 nm

Test method 3 (Hilal Bardakci *et al* 2018**)** [7]**.**
Column: zorbax C18column (4.6 × 150 mm, 3.5 µm).
Mobile phase: Phase A was H2O, the mobile phase B was ACN both containing 0.02% acetic acid.

Gradient program
0 min 15% B; 10 min 20% B; 13 min 20% B; 15 min 25%B; 20 min 30% B; 25 min 30% B; 30 min 50% B.

Mobile phase flow rate: 1.0 mL·min-1; **Sample volume:** 20 µL; **Colum temperature:** 30°C

Detector wavelength: 340 nm

Identification of phenolic compounds through HPLC
Chemicals and Solvents
HPLC Grade Methanol is purchased from Thermo Fisher Scientific India private limited, Mumbai.
HPLC Grade Acetonitrile is purchased from Merck chemicals private limited, Mumbai.
HPLC Grade Water is purchased from Thermo Fisher Scientific India private limited, Mumbai.
Other chemicals used were of Laboratory reagent grade and were purchased from Merck chemicals, Mumbai.

Test method 1 (Gini and Jothi 2018**)** [4]
Column: ZORBAX ODS C18 column (4.6 × 12.5 mm id, 5 µm).
Mobile phase: Solvent A (acetonitrile) and solvent B (0.1% phosphoric acid in water).

Gradient program
92% of solvent B and was held at this concentration for 0–35 min, by 78% of solvent B for the next 35–50 min.

Mobile phase flow rate: 1.0 mL·min-1;
Sample volume: 20 µL; **Colum temperature:** 30°C

Detector wavelength: 280 nm,

Test method 2 (Adriana Skendi *et al* 2017**)** [2]
Column: Ascentis C18 column (250 mm×4.6 mm I.D., 5 µm); **Mobile phase:** (A)1% acetic acid in water, (B) acetonitrile and (C) methanol.

Gradient program
0 min, the A: B: C proportion was 90:0:0; 10 min, 80:4:16; at 25 min, 75:5:20;
30 min, 65:5:30; 31 min, 40:0:60;37 min, 35:20:45;50 min, 20:80:0.

Mobile phase flow rate: 1.5 mL·min-1; **Sample volume:** 20 µL,; **Colum temperature:** 30°C

Detector wavelength: 260 nm

Test method 3 (Nadia Zeghad *et al* 2019**)** [14]
Column: ZORBAX ODS C18 column (4.6 × 12.5 mm id, 5 µm).

Mobile phase: Trifluoroacetic acid 0.05% (v/v) in water to which methanol was added in a linear gradient from 95:5 to 5:95 (v/v) within 70 min.

Mobile phase flow rate: 1.0 mL·min-1; **Sample volume:** 20 µL; **Colum temperature:** 30°C,

Detector wavelength: 280 nm.

Results
The aqueous leaf extracts of *Diospyros chloroxylon* when subjected to HPLC method of Zang *et al* (2010) [23] resulted in the identification of four compounds. Figure 1 shows the chromatogram of leaf extract with 10 peaks. These peaks along with their retention time were compared with the retention time of flavonoid compounds available in literature. Peak number 4, 6, 8 and 10 were corresponded to Apigenin-6, 8-di-C-glucopyranoside (RT: 13.233 min), Kaempferol- 3, 7-di-O-rhamnoside (RT: 20.65 min), Kaempferol-7-O-rhamnoside (RT: 34.583 min) and Isorhamnetin (RT: 44.167) (table 1).
The chromatogram of aqueous leaf extract of *Diospyros chloroxylon* as per the protocol of Sait *et al* (2015) [18] gave seven peaks of which peak number four tallied with Isorhamnetin-3-*O*-dihexoside (RT: 18.33 min) and peak number seven was tallied with 'Isorhamnetin' (RT: 44.23 min) in the literature (Fig 2 & Table 2).
The chromatogram obtained with the leaf aqueous extract as per the protocol of Hilal Bardakci *et al* (2018) [7] is presented in figure 3. In the chromatogram six peaks were observed, of which peak number three (RT 10.56 min) was tallied with 'Scutellarein 7-O-β-D-glucopyranoside' (table 3). Hence, by the application of three different HPLC protocols to the leaf aqueous extracts of *Diospyros chloroxylon*, a total of six flavonoid compounds were identified in the leaf of *Diospyros chloroxylon* that include (i) Apigenin-6, 8-di-C-glucopyranoside (ii) Kaempferol- 3, 7-di-O-rhamnoside (iii) Kaempferol-7-O-rhamnoside (iv) Isorhamnetin (v) Isorhamnetin-3-O-dihexoside and (vi) Scutellarein 7-O-β-D-glucopyranoside (Table 4).
Methanolic leaf extracts of *Diospyros chloroxylon* were also analysed as per the HPLC protocol of Adriana Skendi *et al* (2017) [2]. The chromatogram exhibited nine peaks (figure 4), out of which peak one (Gallic acid RT: 4.5 min), peak three ((−) epigallocatechine RT: 10.0667 min), peak six (Rutin RT: 33.5667 min), peak seven (Quercetin RT:

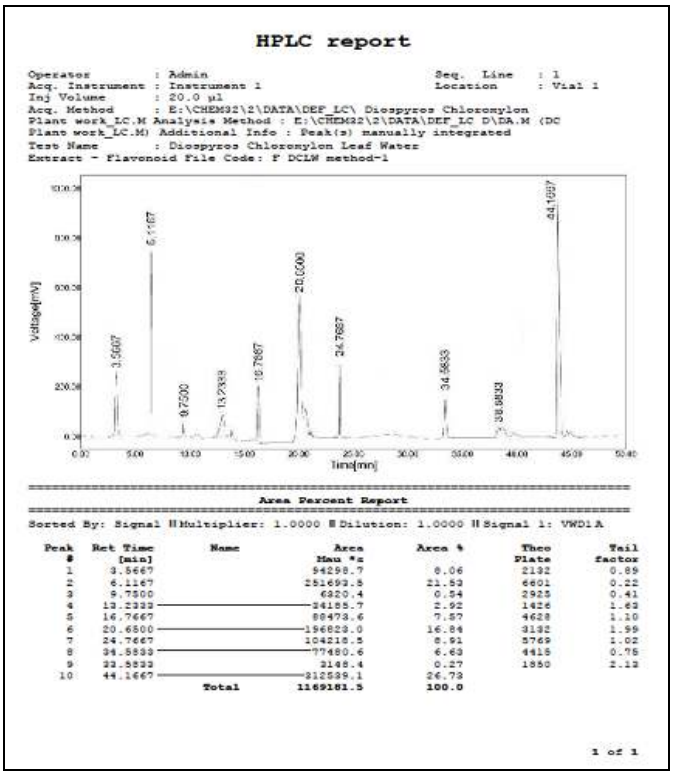
35.6667 min) and peak eight (Kaempferol RT: 37.90 min) were identified with the help of data available in literature (Table 5). Methanolic leaf extracts of *Diospyros chloroxylon* were subjected to HPLC analysis through the protocol developed by Gini & Jothi (2018) [4]. Figure 5 shows the chromatogram with eight (8) peaks out of which peak number two (Gallic acid RT: 5.983 min) and four (Quercetin RT: 13.8 min) were confirmed from the literature data (Table 6). The protocol of Nadia Zeghad *et al* (2019) [14] when used to the methanolic leaf extracts of *Diospyros chloroxylon* resulted in the identification of gallic acid and epicatechin gallate (Fig 6 & Tables 7). Hence, by the application of three different HPLC protocols to the aqueous leaf extracts of *Diospyros chloroxylon*, a total of six phenolic compounds were identified in the leaf that include (i) Gallic acid (ii) quercetin (iii) (-) -epigallocatechine (iv) rutin (v) kaempferol and (vi) epicatechin gallate (Table 8).

The adaption of six different HPLC protocols for aqueous and methanolic leaf extracts of *Diospyros chloroxylon* resulted in the identification of twelve different (phenolic and flavonoid) compounds in the leaf of *Diospyros chloroxylon* Roxb, which include (1) Apigenin-6, 8-di-C-glucopyranoside (2) Kaempferol- 3, 7-di-O-rhamnosid (3) Kaempferol-7-O-rhamnoside (4) Isorhamnetin (5) Isorhamnetin-3-O-dihexoside (6) Scutellarein7-O-β-D-glucopyranoside (7) gallicacid (8) Quercetin (9) Kaempferol (10) Rutin (11) (−) epigallocatechine and (12) Epicatechin gallate

Discussion

The phenolic and flavonoid compounds identified now from leaf of *Diospyros chloroxylon* were earlier found in the other *Diospyros* species. These compounds were considered as more important since they control many biological activities. Madlener *et al* (2007) [12] reported the anticancer activity of gallic acid. Vaquero *et al* (2007) [21] reported antimicrobial activity of quercetin, rutin and gallic acid isolated from different wine varieties. Li & Xu (2008) [10] also reported antimicrobial activity of quercetin. Zhang *et al* (2008) [24] studied the activities of kaempferol and quercetin isolated from straw berry. Kaempferol was found to inhibit the growth of ovarian cancer cell lines (Luo *et al* 2009) [11]. They noticed the inhibitory activity against human oral, colon and prostrate cancer cell lines. The quercetin, kaempferol, rutin and gallic acid isolated from *Zingiber officinale* were shown to inhibit the growth of human breast cancer cell lines (Ghasemzadeh & Jaafar 2011) [3]. Polyphenolics *viz*., epigallocatechine and catechins usually from wine extracts are with anti-cancer activity (Weisburg *et al* 2004) [22]. Catechines are bioactive molecules that provide protection from various ailments (Suzuki *et al* 2005) [20]. The epicatechin gallate, epigallocatechine and gallic acid were previously isolated from highly valued multi-purpose persimmon i.e *Diospyros kaki*. The above catechines were also reported by Achiwa *et al* (1997) [1] as possessed anticancer perspectives against various cancer cell lines. Park *et al* (2006) [15] reported that the catechines, kaempferol and quercetin from *Diospyros kaki* were with free radical scavenging and anti lipid peroxidation activity. Khaled Nabih Rashed *et al* (2013) [9] isolated kaempferol-3-O-α-rhamnoside, kaempferol, gallic acid and quercetin from *Diospyros lotus* and described it as a repertoire of phytochemicals beneficial to human health.

The important flavonoids and phenolic compounds identified now in *Diospyros chloroxylon* underline that it is also highly valued species containing many valuable bioactive molecules. The leaf of *Diospyros chloroxylon* might play a potential role for the preparation of herbal drugs to mitigate oxidative stress created health ailments. Further pharmacological investigations are required to understand the biological activities of the compounds now in *Diospyros chloroxylon*.



HPLC report

Operator : Admin
Acq. Instrument : Instrument 1
Inj Volume : 20.0 µl
Acq. Method : E:\CHEM32\2\DATA\DEF_LC\ Diospyros Chloroxylon
Plant work_LC.M Analysis Method : E:\CHEM32\2\DATA\DEF_LC D\DA.M (DC
Plant work_LC.M) Additional Info : Peak(s) manually integrated
Test Name : Diospyros Chloroxylon Leaf Water
Extract - Flavonoid File Code: F DCLW method-1
Seq. Line : 1
Location : Vial 1

Area Percent Report

Sorted By: Signal ‖ Multiplier: 1.0000 ‖ Dilution: 1.0000 ‖ Signal 1: VWD1 A

Peak #	Ret Time [min]	Name	Area Mau *s	Area %	Theo Plate	Tail factor
1	3.5667		54298.7	8.06	2132	0.89
2	6.1167		251693.8	21.53	6601	0.22
3	9.7500		6320.4	0.54	2923	0.41
4	13.2333		34185.7	2.92	1426	1.63
5	16.7667		88473.6	7.57	4628	1.10
6	20.6500		196823.0	16.84	3132	1.99
7	24.7667		104218.5	8.91	5769	1.02
8	34.5833		77480.6	6.63	4415	0.75
9	38.8833		2148.4	0.27	1850	2.12
10	44.1667		312539.1	26.73		
		Total	1169181.5	100.0		

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Fig 1: HPLC chromatogram of *Diospyros chloroxylon* leaf aqueous extract showing peaks of flavonoid compounds (method-1: Zang *et al* 2010) [23].

Table 1: Flavonoids compounds identified from *Diospyros chloroxylon* Leaf aqueous extract (Method -1: Zang *et al* 2010) [23].

Peak. No	Ret Time [min]	Name identified
1	3.5667	-------
2	6.1167	-------
3	9.7500	-------
4	13.2333	Apigenin-6, 8-di-C glucopyranoside
5	16.7667	-------
6	20.6500	kaempferol- 3, 7-di-O-rhamnoside
7	24.7667	-------
8	34.5833	kaempferol-7-O-rhamnoside
9	33.5833	-------
10	44.1667	Isorhamnetin

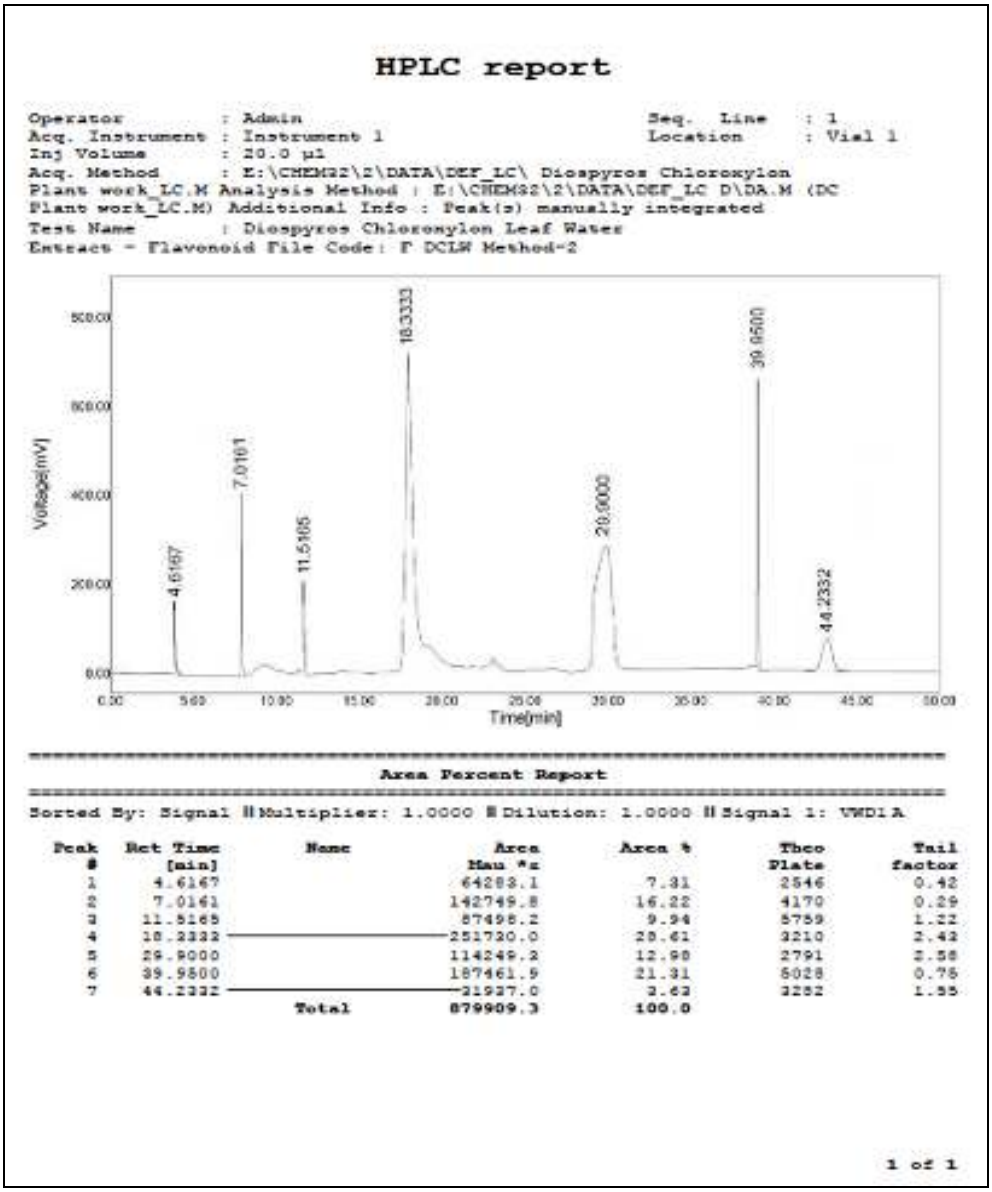
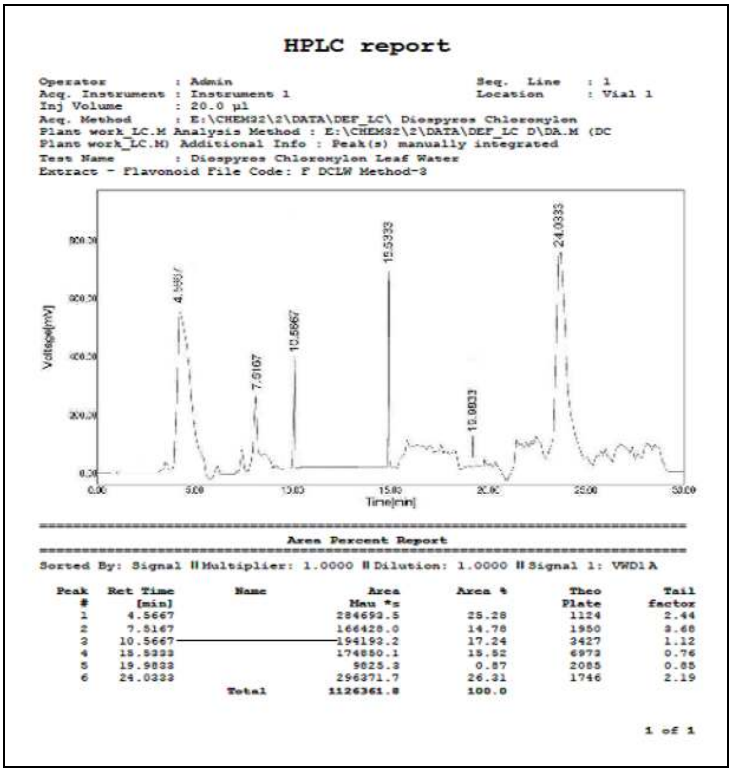


Fig 2: HPLC chromatogram of *Diospyros chloroxylon* leaf aqueous extract showing peaks of flavonoid compounds (method-2: Sait *et al* 2015) [18].

Table 2: Flavonoids compounds identified from *Diospyros chloroxylon* Leaf aqueous extract (Method-2: Sait *et al* 2015) [18]

Peak. No	Ret Time [min]	Name identified
1	4.6167	-------
2	7.0161	-------
3	11.5165	-------
4	18.3333	Isorhamnetin-3-*O*-dihexoside
5	29.9000	-------
6	39.9500	-------
7	44.2332	Isorhamnetin



HPLC report

Operator : Admin
Seq. Line : 1
Acq. Instrument : Instrument 1
Location : Vial 1
Inj Volume : 20.0 µl
Acq. Method : E:\CHEM32\2\DATA\DEF_LC\ Diospyros Chloroxylon
Plant work_LC.M Analysis Method : E:\CHEM32\2\DATA\DEF_LC D\DA.M (DC
Plant work_LC.M) Additional Info : Peak(s) manually integrated
Test Name : Diospyros Chloroxylon Leaf Water
Extract - Flavonoid File Code: F DCLW Method-3

Area Percent Report

Sorted By: Signal ‖Multiplier: 1.0000 ‖Dilution: 1.0000 ‖Signal 1: VWD1 A

Peak #	Ret Time [min]	Name	Area Mau *s	Area %	Theo Plate	Tail factor
1	4.5667		284692.5	25.28	1124	2.44
2	7.5167		166428.0	14.78	1950	3.68
3	10.5667		194192.2	17.24	3427	1.12
4	15.5333		174880.1	15.52	6973	0.76
5	19.9833		9625.3	0.87	2085	0.85
6	24.0333		296371.7	26.31	1746	2.19
		Total	1126361.8	100.0		

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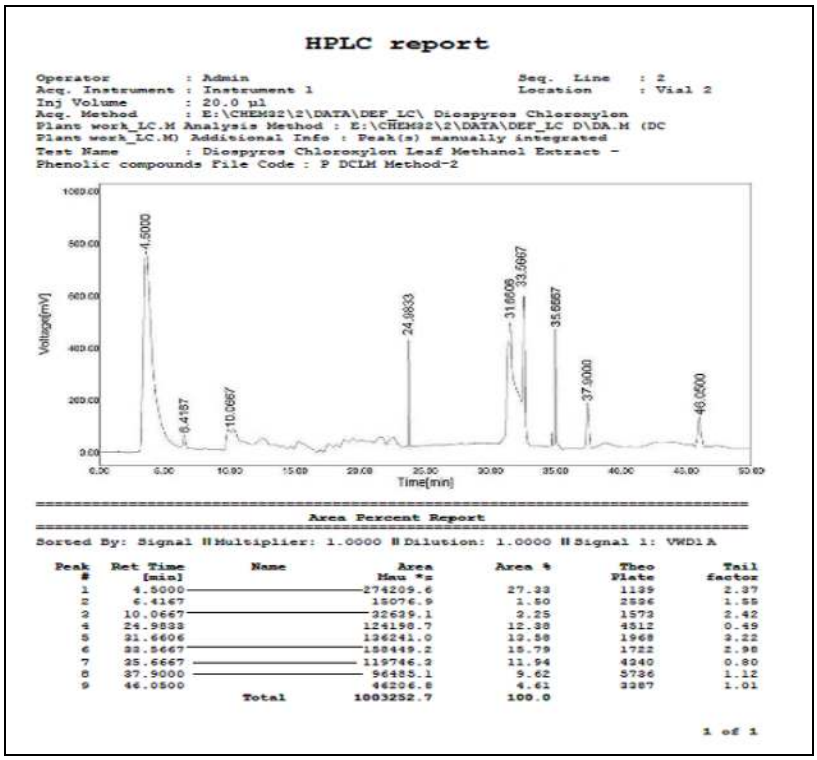
Fig 3: HPLC chromatogram of *Diospyros chloroxylon* leaf aqueous extract showing peaks of flavonoid compounds (method-3: Hilal Bardakci *et al* 2018) [7]

Table 3: Flavonoid compounds identified from *Diospyros chloroxylon* Leaf aqueous extract (Method-3: Hilal Bardakci *et al* 2018) [7]

Peak. No	Ret Time [min]	Name identified
1	4.5667	-------
2	7.5167	-------
3	10.5667	Scutellarein 7-O-β-D-glucopyranoside
4	15.5333	-------
5	19.9833	-------
6	24.0333	-------



HPLC report

Operator : Admin
Seq. Line : 2
Acq. Instrument : Instrument 1
Location : Vial 2
Inj Volume : 20.0 µl
Acq. Method : E:\CHEM32\2\DATA\DEF_LC\ Diospyros Chloroxylon
Plant work_LC.M Analysis Method : E:\CHEM32\2\DATA\DEF_LC D\DA.M (DC
Plant work_LC.M) Additional Info : Peak(s) manually integrated
Test Name : Diospyros Chloroxylon Leaf Methanol Extract -
Phenolic compounds File Code : P DCLM Method-2

Area Percent Report

Sorted By: Signal ‖Multiplier: 1.0000 ‖Dilution: 1.0000 ‖Signal 1: VWD1 A

Peak #	Ret Time [min]	Name	Area Mau *s	Area %	Theo Plate	Tail factor
1	4.5000		274209.6	27.33	1139	2.37
2	6.4167		15076.9	1.50	2596	1.55
3	10.0667		22639.1	2.25	1573	2.42
4	24.9833		124198.7	12.38	4812	0.49
5	31.6606		136241.0	13.58	1968	3.22
6	33.5667		158449.2	15.79	1722	2.98
7	35.6667		119746.2	11.94	4240	0.80
8	37.5000		96455.1	9.62	5736	1.12
9	46.0500		46206.8	4.61	3287	1.01
		Total	1003252.7	100.0		

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Fig 4: HPLC chromatogram of *Diospyros chloroxylon* leaf methanolic extract showing peaks of phenolic compounds (method-2: Adriana Skendi *et al* 2017) [2].

Table 4: Phenolic compounds identified from *Diospyros chloroxolyon* leaf methanol extract by the method of (Method -2) Adriana skendi *et al* 2017 [2].

Peak. No	Ret Time [min]	Name identified
1	4.5000	Gallic acid
2	6.4167	-------
3	10.0667	(−)-epigallocatechine
4	24.9833	--------
5	31.6606	--------
6	33.5667	Rutin
7	35.6667	Quercetin
8	37.9000	Kaempferol
9	46.0500	---------

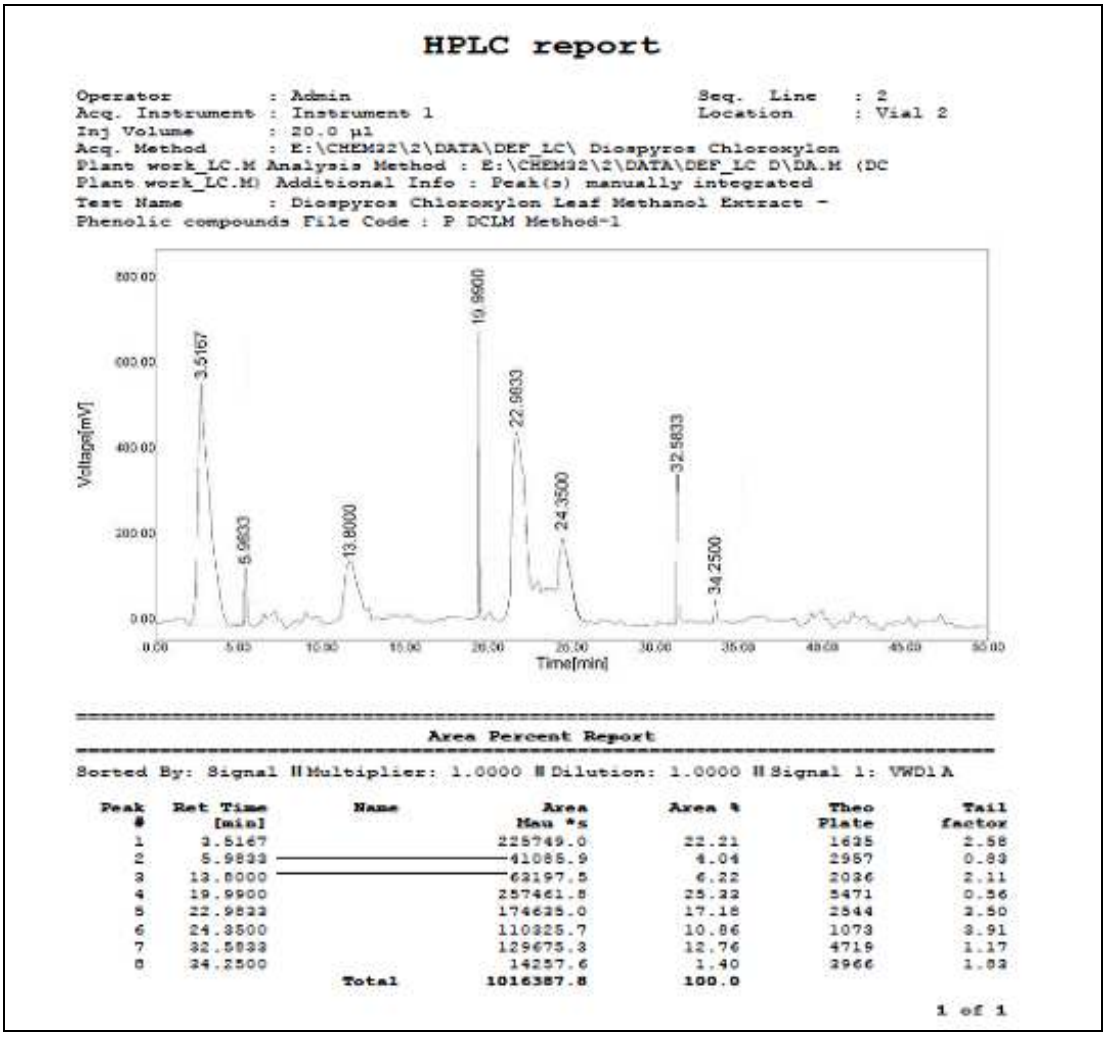
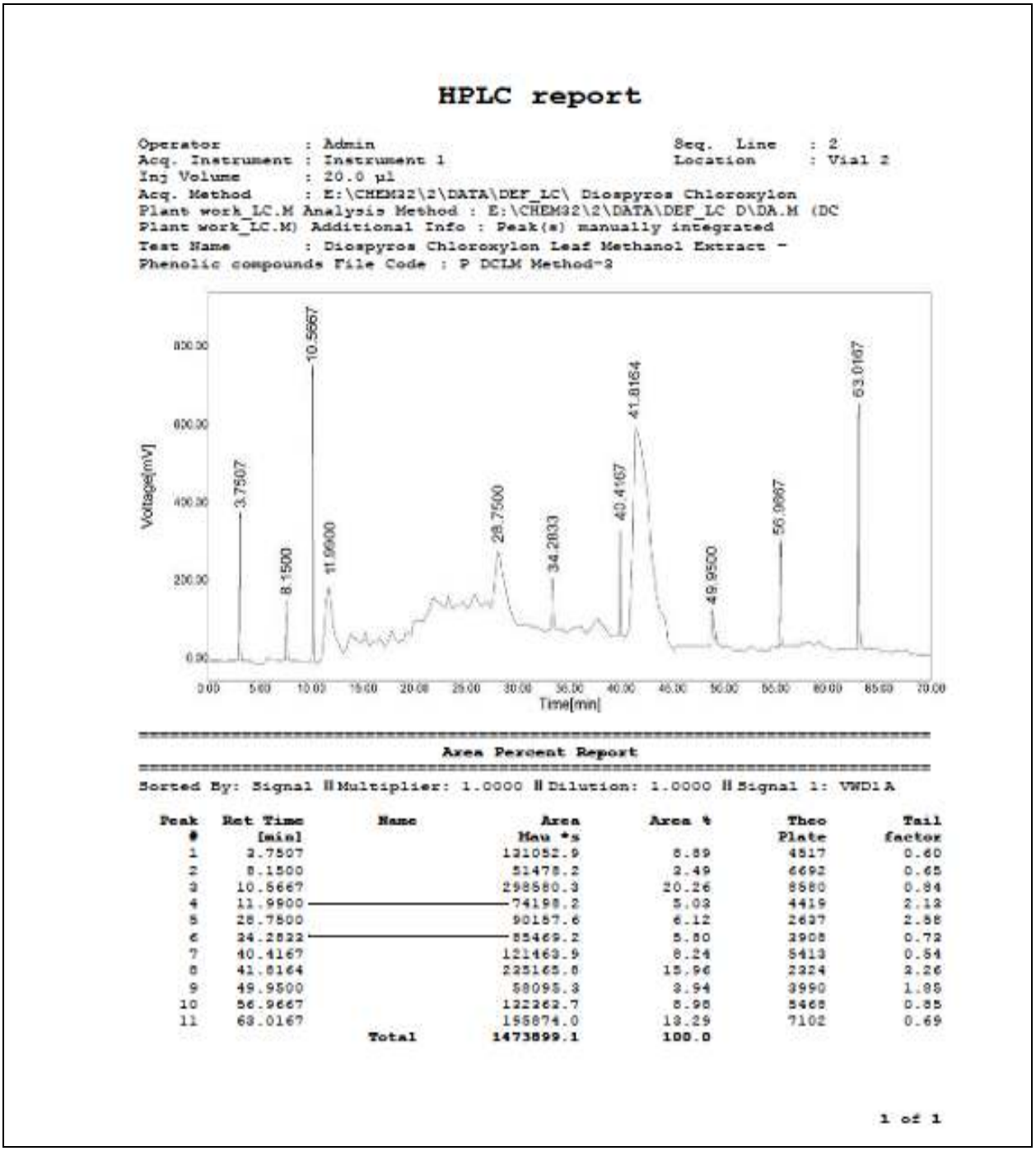


Fig 5: HPLC chromatogram of *Diospyros chloroxylon* leaf methanolic extract showing peaks of phenolic compounds (method-1: Gini TG and Jeya Jothi G 2018) [4].

Table 5: Phenolic compounds identified from *Diospyros chloroxolyon* leaf methanol extract by the method of (Method-1) Gini and Jeya Jothi 2018 [4]

Peak. No	Ret Time [min]	Name identified
1	3.5167	--------
2	5.9833	Gallic acid
3	13.8000	Quercetin
4	19.9900	--------
5	22.9833	---------
6	24.3500	---------
7	32.5833	----------
8	34.2500	----------



HPLC report

Operator : Admin
Acq. Instrument : Instrument 1
Inj Volume : 20.0 µl
Acq. Method : E:\CHEM32\2\DATA\DEF_LC\ Diospyros Chloroxylon
Plant work_LC.M Analysis Method : E:\CHEM32\2\DATA\DEF_LC D\DA.M (DC
Plant work_LC.M) Additional Info : Peak(s) manually integrated
Test Name : Diospyros Chloroxylon Leaf Methanol Extract -
Phenolic compounds File Code : P DCLM Method-2

Seq. Line : 2
Location : Vial 2

Area Percent Report

Sorted By: Signal ‖ Multiplier: 1.0000 ‖ Dilution: 1.0000 ‖ Signal 1: VWD1 A

Peak #	Ret Time [min]	Name	Area Mau *s	Area %	Theo Plate	Tail factor
1	3.7507		121052.9	8.89	4517	0.60
2	8.1500		51478.2	2.49	6692	0.65
3	10.5667		298580.3	20.26	8580	0.84
4	11.9900		74198.2	5.03	4419	2.13
5	28.7500		90157.6	6.12	2637	2.58
6	34.2833		85469.2	5.80	2908	0.72
7	40.4167		121463.9	8.24	5413	0.54
8	41.8164		225165.8	15.96	2324	2.26
9	49.9500		58095.3	3.94	3990	1.85
10	56.9667		122262.7	8.98	5468	0.85
11	63.0167		155874.0	10.29	7102	0.69
		Total	1473899.1	100.0		

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Fig 6: HPLC chromatogram of *Diospyros chloroxylon* leaf methanolic extract showing peaks of phenolic compounds (method- 3: Nadia Zeghad *et al* 2019) [14]

Table 6: Phenolic compounds identified from *Diospyros chloroxolyon* leaf methanol extract by the method of (Method-3) Nadia Zeghad *et al* 2019 [14]

Peak. No	Ret Time [min]	Name identified
1	3.7507	-------
2	8.1500	-------
3	10.5667	-------
4	11.9900	Gallic acid
5	28.7500	-------
6	34.2833	Epicatechin gallate
7	40.4167	-------
8	41.8164	-------
9	49.9500	-------
10	56.9667	-------
11	63.0167	-------

Table 7: HPLC identification of different flavonoid compounds in *Dispyros chloroxylon* Roxb leaf aqueous extract (consolidated from Zang *et al* 2010 [23]; Sait *et al* 2015 [18]; Hilal Bardakci *et al* 2018) [7].

Peak. No	Name of the flavonoid compound	RT in Min
Method-1 (Zang *et al* 2010) [23]		
4	Apigenin-6, 8-di-C-glucopyranoside	13.233
6	kaempferol- 3, 7-di-O-rhamnoside	20.6500
8	kaempferol-7-O-rhamnoside	34.5833
10	Isorhamnetin	44.1667
Method-2 (Sait *et al* 2015) [18]		
4	Isorhamnetin-3-O-dihexoside	18.333
7	Isorhamnetin	44.2332
Method-3 (Hilal Bardakci *et al* 2018) [7]		
3	Scutellarein 7-O-β-D-glucopyranoside	10.5667

Table 8: HPLC identification of different phenolic compounds in *Dispyros chloroxylon* Roxb. leaf methanolic extract (consolidated from Gini and Jeya Jothi 2018 [4]; Adriana skendi *et al* 2017 [2]; Nadia Zeghad *et al* 2019) [14]

Peak. No	Name of the phenolic compound	RT in Min
Method-1 (Gini TG and Jeya Jothi G 2018) [4]		
2	Gallic acid	5.9833
3	Quercetin	13.8000
Method-2 (Adriana Skendi *et al* 2017) [2]		
1	Galic acid	4.5000
3	(−)-epigallocatechine	10.0667
6	Rutin	33.5667
7	Quercetin	35.6667
8	Kaempferol	37.9000
Method-3 (Nadia Zeghad *et al* 2019) [14]		
4	Gallic acid	11.9900
6	Epicatechin gallate	34.2833

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