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Isolation, Structural Elucidation, Of Flavonoids and Phenolic Constituents from Stem of Fiscus Cordata Thunb. Subsp, Salicifolia (vahl).

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Abstract:

The current study was to deal the isolation and identification of secondary metabolites from the stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl). The hespirdin was the highest concentration of flavonoid and E-vanillic acid was the highest concentration of phenolic acid in the stem extract by using HPLC. The ethanolic extract of the plant stem was fractionated and separated to obtain the isolated compounds by different chromatographic techniques. Structures of these compounds were elucidated by R_f-values, UV, ^{1}H-NMR, ^{13}C-NMR spectral analysis and compared with the literature data. Seven secondary metabolites were isolated from stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl); five flavonoid compounds were identified as 5, 7 dihydroxy flavanone, pinocembrin, rutin, apigenin, quercetin, luteolin-7-*O*-glucoside and two phenolic compounds were identified as gallic acid and ferulic acid.

Keywords: *Moraceae, Ficus cordata* Thunb. subsp. *salicifolia* (Vahl), flavonoids, phenolic compounds.

Introduction:

Several researches of natural product have been focused on phytochemical products of family Moraceae. The Moraceae often called the mulberry family or fig family. It is a family of flowering plants comprising about 50 genera and over 1500 species. Most are widespread in tropical and subtropical regions, less so in temperate climates **(Mahbubur Rahman and Khanom, 2013)**. *Ficus* is one of the

largest genera of angiosperms from the mulberry family with more than 800 species of trees, shrubs, hemi epiphytes, climbers and creepers in the tropical and subtropical region all over the world **(Frodin, 2004)**. The *Ficus* genus is one of the most populous in number of species of all plant genera. Phytochemical investigations of some *Ficus* species revealed that, phenolic compounds are their major components **(Abdel-Hameed, 2009)**. *Ficus* species have promising pharmacological activities **(Salem *et al*., 2013)**. Moreover, *Ficus* species are rich source of polyphenolic compounds, flavonoids which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases **(Sirisha *et al*., 2010)**. Leaves of *F. bengalensis* contain flavonols that are responsible for its antioxidant effects. These flavonols include quercetin-3-galactoside and rutin. Stem bark of *F. bengalensis* also contains bengalenosides that is, glycosides or flavonoids, 5, 7 dimethyl ether of leucoperalgonidin -3-*O*-*α*-L-rhamnoside and 5, 3 dimethyl ether of leucocyanidin 3-*O*-*β*-D- galactosyl cellobioside, and 5, 7, 3 trimethoxy leucodelphinidin 3-*O*-*α*-L-rhamnoside **(Vikas and Vijay, 2010)**. Phenolic acids such as 3-*O*- and 5-*O*-caffeoylquinic acids, ferulic acid, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, psoralen, bergapten, and organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric) have been isolated from the water extract of the leaves of *F. carica* L. **(Oliveira *et al*., 2009)**. The nutritive value of plant leaves is higher than plant stem due to the highest carbohydrates and fat accompanied by enough protein content of plant leaves, therefore, the plant may be has some nutritional value, Seventeen free & protein amino acids with different ranges of concentrations were detected in plant leaves and stem by using Amino Acid Analyzer. The separation of free and combined sugars contents in leaves and stem was achieved using High Performance Liquid Chromatography (HPLC), which revealed the presence of

eleven free sugars and nine combined sugars **(Ahmed *et al.*, 2017)**. Also, the preliminary phytochemical screening of leaves and stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) plant proved the presence of a large variety of phytochemical constituents such as glycosides and/or carbohydrates, saponins, alkaloids, flavonoids, tannins, sterols and/or terpenes, phenolics and amino acids were detected in plant leaves and stem. These phytochemical constituents are of physiological importance and possess hypolipidemic, anti-tumor or stimulating properties which can reduce the risks of cardiovascular disease and cancer**,** the methanolic extract of the stem of *Ficus cordata* contains total flavonoids and phenolics higher than its leaves and the methanolic extract of stem *of Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) possess antioxidant effects due to its high content of phenolics and flavonoids which reduced the oxidative stress in testes of rats intoxicated by lead **(Ahmed *et al.*, 2017)**. Thus, flavonoid and phenolic compounds are isolated from the stem of the plant in this study.

Materials and Methods:

1. Plant Material:

A sample of *Ficus cordata* subsp *salicifolia* stem was collected from Elba mountain habitat in the remotest south eastern corner of Egypt during the period of investigation in February 2014. Identification of the plant was confirmed by the Herbarium, at Desert Research Center. The samples were separately air-dried, powdered, weighted and kept in tightly-closed amber coloured glass containers and protected from light at low temperature.

2. Qualitative and Quantitative Determination of Phenolics and Flavonoids using HPLC:

Qualitative and quantitative determination of phenolics and flavonoids of stem extracts using HPLC was carried out according to the method described by **Mattila *et al.*, (2000).**

3. Investigation of Flavonoids and Phenolics:

3.1. General experimental procedure:

^{1}H-NMR and ^{13}C-NMR spectra were obtained on Bruker AMX-400, Avance 400, and Avance 300 spectrometers (Bruker, Rheinstetten, Germany) with standard pulse sequences operating at 400, 300 MHz in ^{1}H-NMR and 100, 75 MHz in ^{13}C-NMR. Chemical shifts are given in δ values (ppm) using DMSO as the internal standard. UV spectra were recorded with Shimadzu UV-1601 (Shimadzu, Tokyo, Japan). Column chromatography (CC) was carried out on silica gel (E.Merck) 70-230 mech and Sephadex LH- 20 (Fluka, Pharmazia, Uppsala, Sweden); thin layer chromatography TLC (Fluka) with fluorescent 254nm (20 x 20cm) and paper chromatography (PC) was carried out on Whatman No. 1 and 3 mm paper. The following solvent systems were used: AcOH/H_2O (15:85); BAW (nBuOH/HOAc/H_2O, 4:1:5); $CHCl_3$/ EtOAc 7:3 v/v; $CHCl_3$/ EtOAc 7:3 v/v; $CHCl_3$/ EtOAc 8:2 v/v; $CHCl_3$/ EtOAc 3:7 v/v; $CHCl_3$/ MeOH 9:1 v/v; $CHCl_3$/ EtOAc 1:9 v/v; $CHCl_3$/ MeOH/H_2O 9:1:3; $CHCl_3$/ EtOAc 4:6 v/v; EtOAc/MeOH 3:7 v/v; EtOAc/MeOH 8:2 v/v; MeOH / H_2O (1:1v/v); Pet. ether / $CHCl_3$ 6:4 v/v and toluene/ EtOAc:HCOOH, 5:4:1 v/v.

3. 2. Extraction, isolation and purification:

Dry powder (1500 gm) of *Ficus cordata* subsp *salicifolia* stem subjected to successive extraction using different organic solvents according to their polarity starting by petroleum ether followed by chloroform then ethyl acetate and ending by methanol and purified according to procedures reported by **Agrawal *et al*., (2012)**. Each time before employing the solvent of higher polarity, plant powder was dried. Each extract was then concentrated using rotary vaccum evaporator at 40-50^{o}C under vacuum and dried residue was collected in an opaque glass bottles

to produce 4 main fractions for further studies. Petroleum ether fraction (73 gm); subjected to preparative paper chromatography (3MM) using system BAW (4:1:5) for 24 hours, given one major band. The band was eluted separately with hot MeOH / H_2O (1:1v/v), which applied to TLC (14 sheet), in (Pet. ether / $CHCl_3$ 6:4 v/v) given one main blue band, scratched, then soaked in pure ethanol to isolate compound 1 (87.1 mg). Chloroform fraction (68.2 gm); was applied on the top of silica gel column (3 cm X 120 cm, 130 gm silica gel), 20 drops per minute and 50 ml per fraction, started from chloroform and increased in polarity, to produced two main subfractions, first one eluted with a mixture of ($CHCl_3$/ EtOAc 7:3 v/v) and was subjected to preparative TLC (15 sheet $CHCl_3$/ MeOH 9:1 v/v) to obtain one main band, scratched, then soaked in pure ethanol to isolate compound 2 (72.3 mg), the second fraction eluted with a mixture of (EtOAc /MeOH 8:2 v/v), then was applied on the top of silica gel sub column (2cm X 80cm, 50 gm silica gel, 20 drops per minutes and 50 ml per fraction), where one main major spot eluted with a mixture of ($CHCl_3$/ EtOAc 4:6 v/v), then, applied to preparative TLC (10 sheet, eluted with $CHCl_3$/ MeOH 9:1 v/v) to isolate compound 3 (42 mg). Ethyl acetate fraction (65.4 gm); was applied on the top of the silica gel column (5cm X 100cm, 110 gm silica gel, 20 drops per minute and 50 ml per fraction), column started from $CHCl_3$ and increased in polarity. Two main sub-fractions obtained. First one eluted with a mixture of ($CHCl_3$/EtOAc 3:7 v/v), applied to prep. TLC (12 sheet), where one main yellow band scratched, then soaked in pure ethanol to obtained compound which further purified by loading in Sephadex column to obtained one pure yellow compound 4 (55 mg). The second sub-fraction eluted with a mixture of (ethyl acetate/ MeOH 3:7 v/v) was applied on the top of silica gel column (2cm X 70cm, 40 gm silica gel, 20 drops per minutes and 50 ml per fraction) started from $CHCl_3$ and increased in polarity, one main compound eluted with a mixture

of ($CHCl_3$/ EtOAc 1:9v/v), applied to preparative TLC (13 sheet), one main yellow band obtained. It scratched, then soaked in pure ethanol to isolate compound 5 (46.1 mg). Methanol fraction (102.4 gm); was applied on the top of the silica gel column (7cm X 180cm, 200 gm silica gel, 20 drops per minute and 50 ml per fraction) started from $CHCl_3$ and increased in polarity, to produced two main sub fractions, the first one eluted with a mixture of ($CHCl_3$: EtOAc 8:2 v/v), applied to preparative TLC (15 sheet) to obtain one main yellow band, this band scratched, then soaked in pure ethanol to obtained one main yellow compound, further purified by loaded on sephadex column to isolate compound 6 (72.6 mg). The second sub-fraction eluted with a mixture of (EtOAc/MeOH 3:7 v/v) was subjected to preparative paper chromatography (3MM) using system BAW (4:1:5) for 24 hours, given one major band, reloaded on preparative TLC (9 sheet) in system (EtOAc / MeOH 3:7 v/v) to obtained one main yellow band, scratched, then soaked in pure ethanol to isolate one main yellow compound 7 (53.1 mg).

4. Results and Discussion:

4.1. Phenolic compounds of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) stem using HPLC: The results recorded in table (1) and figures (1,2) showed the types and concentrations of flavonoids and phenolic acids present in stem extract of *F. c.* (Vahl) which revealed that; the highest concentration of flavonoid was that of hespirdin (1106.04 ppm) followed by luteolin 6-arabinose-8-glucose (722.03 ppm), while the lowest concentration was apigenin (9.97 ppm) as indicated in table, (1) and the highest concentration of phenolic acid was E-vanillic acid (3178.57 ppm) followed by ellagic acid (1731.7 ppm), while the lowest concentration was that of 4- amino benzoic acid (4.79 ppm) as indicated in table (1).

4.2. Flavonoids and phenolic compounds: Seven compounds (C_1- C_7) were isolated from the stem part of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl). The isolated compounds undergo conventional chemical and spectroscopic methods of analysis (UV, ^{1}H-NMR and ^{13}C-NMR) as well as chromatography to elucidate their chemical structures.

Compound (C_1): R_f value (x100) 0.93 in (BAW), 0.57 in (HOAc-15%); faint blue, soluble in acetone and methanol and insoluble in water; UV spectral data λ_{max} (nm): MeOH: 272, 335+ NaOMe: 275, 345; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): δ 7.09 (s, 2H, H-2 and H-6); From this data and by comparing with those published before **(Silverstein *et al*., 1981).** C_1 was identified as 3, 4, 5 trihydroxy benzoic acid (gallic acid).

Compound (C_2): R_f value (x100) 0.93 in (BAW), 0.57 in (HOAc-15%); yellow powder, soluble in methanol; UV spectral data λ_{max} (nm): MeOH: 282 (major band), 312 (Flavanone) + NaOMe: 280, 308 (sh), 314 (Free OH at 5) + NaOAc: 285 (sh), 316 (Free OH at 7),+ NaOAc/H_3BO_3: 301, 314 (sh),+ $AlCl_3$: 299, 325 (Free OH at 5) + $AlCl_3$/HCl: 304, 325 (Absence ortho-dihydroxyl groups); ^{1}H-NMR (400 MHz, DMSO-d6) δ (ppm): δ 7.28 (m, 5H, H of ring B), 6.3 (d, H, J= 3.1, H-8), 6.2 (d, H, J=2.6, H-6), 5.4 (dd, H, J=16.1, 5.11, H-2), 2.8 (dd, cis and trans 2H, J= 17.1, 4.8, H-3). Thus, from this data and by comparing with published data **(Mabry *et al.*, 1970)**, C_2 was identified as 5, 7 dihydroxy flavanone (Pinocembrin).

Compound (C_3): R_f value (x100) 0.65 in (BAW), 0.88 in (HOAc-15%); soluble in acetone and methanol and insoluble in water. Color of spot: blue in UV, yellow in UV/NH_3, yellow in UV/$AlCl_3$; UV spectral data λ_{max} (nm): MeOH: 272, 300 (sh), 335+ NaOMe: 250 (sh), 290, 245; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): δ8.03 (1H, d, J= 17Hz, H-1'), 7.54 (1H, d, J=7.5 Hz, H-2), 7.34 (1H, dd, J=7.5 & 2.5 Hz, H-6), 7.05 (1H, d, J=2.5 Hz, H-5), 6.25 (1H, d, J=17Hz, H-2'), 4.03 (3H, s, -OCH_3). From this obtained data and by comparing with those published before **Silverstein *et al*., (1981)**, C_3 was identified as 4-hydroxy-3-methoxy cinnamic acid (ferulic acid).

Compound (C_4): R_f value (x100) 0.45 in (BAW), 0.71 in (HOAc-15%); yellow powder, soluble in methanol; color of spot: yellow in UV, yellow in UV/NH_3, yellow in UV/$AlCl_3$; UV spectral data λ_{max} (nm): MeOH: 250, 329sh, 368, (Flavonol) + NaOMe: 270, 329 (sh), 410 (Free OH at 4`)+ NaOAc: 263, 355 (sh), 382 (Free OH at 7) + NaOAc/H_3BO_3: 264, 298, 433 (ortho-hydroxy group at B ring) + $AlCl_3$: 270, 433 (Free OH at 5) + $AlCl_3$/HCl: 271, 331(sh), 430 (ortho-

dihydroxy group at B ring). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): δ 7.57 (1H, d, J=2.1 Hz, H-2'), 7.54 (1H, dd, J=9, 2.1 Hz, H-6'), 6.89 (1H, d, J=9.0 Hz, H-5'), 6.40 (1H, d, J=2.1 Hz, H-8), 6.20 (1H, d, J=2.1 Hz, H-6), 5.35 (1H, d, J=7.5 Hz, anomeric H-1``, glucose), 4.40 (H, broad singlet, anomeric H-1```, rhamnose), 3.25-3.45 (m, the rest sugar of glucose and rhamnose), 1.0 (3H, d, J=6.3 Hz, CH_3-rhamnose). From the previously mentioned data and by comparing with those published before **(Mabry *et al*., 1970)**, C_4 was identified as rutin (Quercetin-3-O-α-L-rahamnopyranosyl-(1→6)-β-D-glucopyranoside).

Compound (C_5): R_f value (x100) 0.81 in (BAW), 0.13 in (HOAc-15%); amorphous yellow powder, soluble in methanol, color of spot: yellow in UV, yellow in UV/NH_3, bright yellow in UV/ $AlCl_3$; UV spectral data λ_{max} (nm): MeOH: 275, 342 (Flavone),+ NaOMe: 277, 378 (Free OH at 4'),+ NaOAc: 287, 283 (sh), 322 (Free OH at 7) + NaOAc/H_3BO_3: 264, 298, 433 (*ortho*-hydroxy group at B ring) + $AlCl_3$: 270, 433 (Free OH at 5) + $AlCl_3$/HCl: 292, 346 (*ortho*-hydroxy group at B ring). ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 7.8 (2H, d, J=8.8 Hz, H-2` and H-6`), 6.8 (2H, d, J=8.8 Hz, H-3`and 5`), 6.4 (1H, d, J=2.57 Hz, H-8), 6.5 (1H, s, H-3), 6.1 (1H, d, J=2.57 Hz, H-6). Thus from the previous analysis of the data and by comparing with published data **(Mabry *et al*., 1970)**, C_5 could be identified as 4′, 5, 7-trihydroxyflavone (Apigenin).

Compound (C_6): R_f value (x100) 0.75 in (BAW), 0.21 in (HOAc-15%); amorphous, yellow powder, soluble in methanol; color of spot: yellow in UV, yellow in UV/NH_3, yellow in UV/ $AlCl_3$; UV spectral data λ_{max} (nm): MeOH: 263, 306 (sh), 370 (Flavonol) + NaOMe: 297, 331, 432+ NaOAc: 273, 305 (sh), 380) + NaOAc/H_3BO_3: 266, 325 (sh), 390 + $AlCl_3$: 280, 439 (Free OH at 5) + $AlCl_3$/HCl: 280, 330 (Free *ortho*-dihydroxy group at B ring). ^{1}HNMR (400 MHz, DMSO-d_6) δ (ppm): 7.8 (2H, d, J=8.8 Hz, H-2`and H-6`), 6.8 (2H, d, J=8.8 Hz, H-3`and 5`), 6.4 (1H, d, J=2.57 Hz, H-8), 6.5 (1H, s, H-3), 6.1 (1H, d, J=2.57 Hz, H-6). From the previously mentioned data and by comparing with those published before **(Mabry *et al*., 1970),** C_6 identified as quercetin.

Compound (C_7): R_f value (x100) 0.48 in (BAW), 0.16 in (HOAc-15%); yellow powder, soluble in methanol, color of spot: Yellow in UV, yellow in UV/NH_3, Faint brown in UV/$AlCl_3$; UV spectral data λ_{max} (nm): MeOH: 255, 281 (sh), 341 (Flavone) + NaOMe: 262, 298 (sh), 392 (Free OH in ring A & B) + NaOAc: 259, 402 (no free OH at 7), + NaOAc/H_3BO_3: 258, 292 (sh), 372+ $AlCl_3$: 272, 302 (sh), 420 (free OH at 5),+ $AlCl_3$+ HCl: 272, 298 (sh), 383 (*ortho* di-OH group in ring B). ^{1}HNMR (400 MHz, DMSO-d6) δ (ppm): 7.39 (dd, J = 7.6, 2Hz, H-6'), 7.3 (d, J = 2.5 Hz, H-2'), 6. 8 (d, J=8.4 Hz, H-5'), 6.6 (s, H-3), 6.7(d, J=2 Hz, H-8), 6.38 (d, J=2Hz, H-6) and 5.02 (d, J=7.6 Hz, H-1" of *β*-D-glucose), 3.25-4 (m, remaining sugar proton). Thus from the previous analysis of the data and by comparing with

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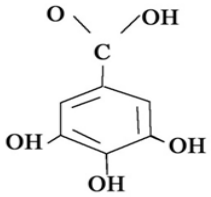
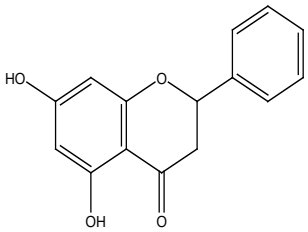
published data **(Mabry *et al.*, 1970)**, C_7 could be identified as luteolin-7-*O*-glucoside (Cinaroside).

Structures of isolated compound:

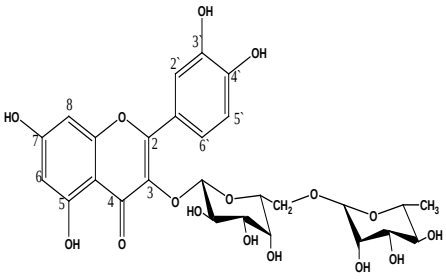


C_1

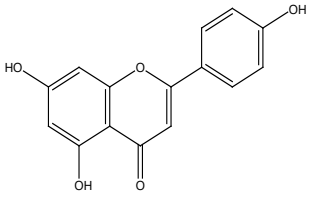


C_2

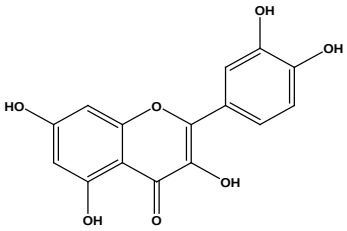
C_3



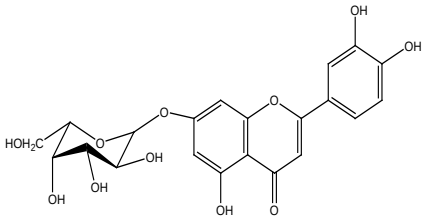
C_4



C_5



C_6



C_7

Ficus species are rich source of polyphenolic compounds and flavonoids which are responsible for strong antioxidant properties that help in prevention and therapy of various oxidative stress related diseases **(Sirisha *et al*., 2010).** In this study, flavonoid and phenolic compounds; gallic acid (C_1), 5, 7 dihydroxy flavanone (C_2), ferulic acid (C_3), rutin (C_4), apigenin (C_5), quercetin (C_6) and luteolin-7-O-glucoside (C_7) were isolated from the stem extract of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl), each of them has antioxidant activity **[Metodiewa *et al*., (1997); Lakhanpal and Rai, (2007); Srinivasan *et al*., (2007); Zielińska *et al*., (2010); Makboul *et al*., (2014) and Badhani *et al*., (2015)].** This result was cofirmed with **Ahmed *et al*., (2017)** they resulted that, stem extract of *Ficus cordata* Thunb. Subsp. *salicifolia* (Vahl) possess antioxidant effects due to its high content of phenolics and flavonoids which reduced the oxidative stress in testes of rats intoxicated by lead. The flavonoid and phenolic compounds, which were separated from the stem part of *Ficus cordata* Thunb. subsp. salicifolia (Vahl) have an important medicine, compound C_1 was used as Hepatoprotective **(Seyed *et al*., 2013),** C_2 was used as analgesic, anti-mutagenic **(Joseph *et al*., 2008; Murti and Mishra, 2014**), C_3 used as anti-diabetics & anti-cancer **(Barone *et al*., 2009),** C_4 used as antimicrobial & anti-inflammatory **(Rigano *et al*., 2006 & Antunes-Ricardo *et al*., 2015)**, C5 used as anti-inflammatory & anxiolytic **(Zhou *et al*., 2017; Jeong *et al*., 2009)**, C_6 used as anti-diabetics & anti-antimicrobial **(Costantino, 1999; Rigano et al., 2006)**, C_7 used as anti-inflammatory **(Park and Song, 2013)**. Thus, further investigations are required in order to make optimal use of this plant.

Conclusion:

The stem extract of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl), is good source of flavonoids and phenolics which has an important antioxidant properties.

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Table (1): Flavonoid and Phenolic constituents (ppm) in stem of *Ficus cordata* Thunb. subsp. *salicifolia* (Vahl) using HPLC.

Flavonoid type	RT	Ppm	Phenolic type	RT	(ppm)
Luteo.6-arabinose-8-glucose	9.494	722.03	Pyrogallol	6.943	494.88
Luteo.6-glucose-8-arabinose	10.711	93.38	Gallic acid	7.041	7.90
Apig.6-arabinose-8-glactose	11.657	177.39	4-amino benzoic acid	8.262	4.79
Apig.6-rhamnose-8-glucose	12.018	64.00	Protocatchuic acid	8.403	168.63
Apig.6-glucose-8-rhamnose	12.183	136.82	Catechin	8.633	25.49
Luteo.7-glucose	12.320	78.01	Catechol	9.000	83.60
Narengin	12.360	105.69	Epi-catechin	9.713	45.12
Hespiridin	12.521	1106.04	*P*-OH-benzoic acid	9.774	104.69

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Rutin	12.610	66.96	Caffeic acid	9.869	55.60
Apig.7-O-neohespiroside	13.167	126.90	Chlorogenic acid	10.100	63.81
Kamp.3,7-dirhamnoside	13.282	73.57	Vanillic acid	10.187	85.54
Quercetrin	13.470	44.24	*P*-Coumaric	11.542	18.25
Quercetin	14.976	27.28	Ferulic acid	11.867	65.35
Naringenin	15.020	11.76	Iso-ferulic acid	12.164	22.10
Acacetin neo. rutinoside	15.180	188.47	E-vanillic acid	12.233	3178.57
Hespirtin	15.347	93.05	Alpha-coumaric	13.190	51.11
Kampferol	16.244	51.37	Benzoic acid	13.287	522.83
Rhamnetin	16.426	15.17	Ellagic acid	13.400	1731.70
Apegnin	16.514	9.97	3,4,5-methoxycinnamic acid	14.137	254.16
Apegnin-7-glucoside	17.190	21.87	Coumarin	14.447	48.22
Acacetin	18.914	184.64	Cinnamic acid	15.317	6.00

RT=Retention time

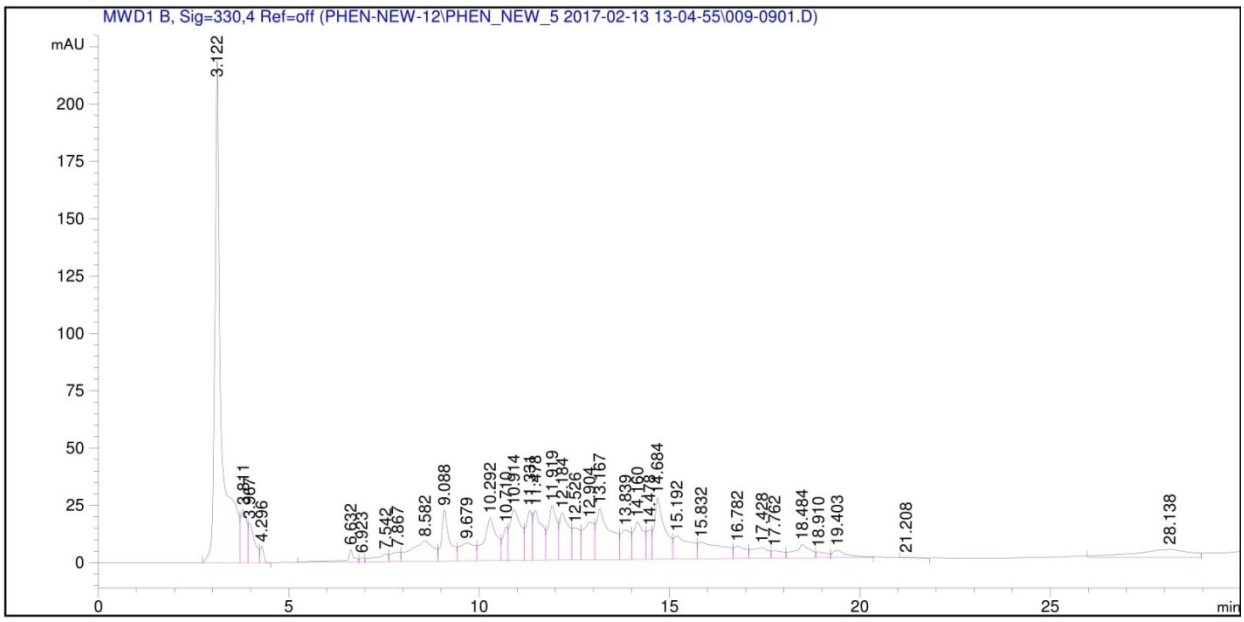

Fig. (1): HPLC chromatogram of flavonoids stem extract.

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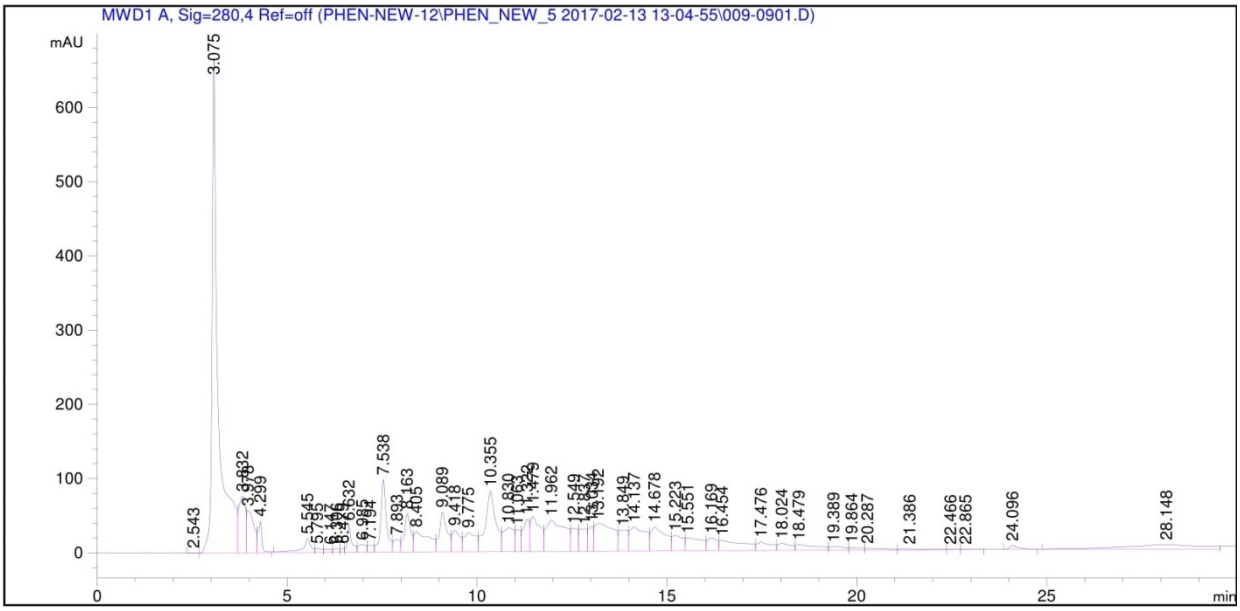

Fig. (2): HPLC chromatogram of phenolic constituents stem extract.