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Polyphenols profile and antioxidants capacity of Verbenaceae species from Burkina Faso

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Abstract

Verbenaceae species are widely used in the traditional medicine to treat tumors, liver pathologies, hypertension and nervous hepatitis. The aim of this present study was to evaluate phenols and flavonoids chromatographic profile of 5 Verbenaceae species directed against antioxidant activities. 3 types of extracts were used in this evaluation such as decoction, acetone and EtOH-H_2O extracts. Chemical composition was evaluated using aluminum chloride, Folin-Ciocalteu and HPLC-DAD methods. 3 antioxidant methods were used such as total antioxidant (TAC), iron reducing power (IRP) and radical scavenging activity (DPPH*). Principal component analysis (PCA) was used to research difference inside chemical polyphenolic compounds. High performance liquid chromatography with photodiode matrix, allowed us to reveal 56 polyphenolic compounds in different parts of the plant. The most represented compounds were Luteolin (7), Chlorogenic acid (5), Genkwanin (4), Rosmarinic acid (4), and Scutellarein-6,4'-dimethylether (4). The best total phenolsand flavonoids content was obtained with EtOH-H_2O extract (111,255 mg GAE.g^{-1} of dry tissue) and (4.42 mg QE. g^{-1} of dry tissue), respectively. The best antioxidant activitiesweregiven in the order of EtOH-H_2O > acetone and decoction extracts with *Stachytarpheta angustifolia*. The specific and qualitative profiles of each species allowed their separation or discrimination with principal component analysis. The relevant antioxidant properties, suggesting that different parts of the plant of Verbenaceae are an important source of natural antioxidants.

Key- words:Verbenaceae; phenol acids; chromatographic profils; antioxidants; compounds

Introduction

The plant secondary metabolites are an important part for the development of plants, these being the most important for their chemical properties and for their high content of active compounds used in traditional medicine (Pardo *et al.*, 2011).

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According to the World Health Organization (WHO, 2002), traditional medicine is largely more available than modern medicine. Nowadays, approximately 50 drugs result from the tropical plants (Gurib-Fakim, 2006; Bangou *et al.*, 2012a). Fifty percent of the products prescribed in several countries of Europe and America are natural products or their derivatives (Gurib-Fakim, 2006; Bangou *et al.*, 2012a). Among these plant families, the species of that of Verbenaceae belong to the most used (Bangou *et al.*,

2012a; Bangou *et al.*, 2012b). These plants are widely used in the treatment of diseases such as inflammatory diseases, hypertension, high blood pressure, tumors, liver pathologies, nervous hepatitis, cancer, tooth pain and skin diseases (Sawadogo*et al.*, 2015; Bangou *et al.*, 2012b; Pascual *et al.*, 2001; Nacoulma, 1996). These diseases are becoming nowadays a threat to human health (Nacoulma, 1996). For example, cancer disease had caused approximately 14 million new cases in 2012 and was responsible for the death of 8.8 million in 2015 (World Cancer Report, 2014).

Oxidative stress results from imbalance between the oxidization and the free radicals trapping (Bangou *et al.*, 2017). Former investigation was showed that plants secondary metabolites are highly important source of antioxidants in the benefits of human health (Lin *et al.*, 2016; Ozcan*et al.*, 2014; Saxena *et al.*, 2012; Cai *et al.*, 2004). It's indicated that the consumption or intake of these natural antioxidants, allow a healthier life (Ozcan et al., 2014; Naqvi et al., 2013; Khan *et al*., 2012; Saxena et al., 2012).

Five well-known plants species such as *Duranta erecta* L, *Gmelina arborea* Roxb, *Lantana camara* Moldenke, *Stachytarpheta angustifolia* (Mill) Vahl and *Tectona grandis* L were subject of our study. Certain number of molecules hasbeen already isolated in these plants. Iridoidgycoside 6-*O*-(2,3-dibenzoyl)-α-l-rhamnopyranosylcatalpol (IG), isoxazole alkaloid, iridoid, phenylpropanoid glycosides, lignans and verbascoside from *G. arborea* and *T. grandis* (Bangou *et al.*, 2012b; Dhakulkar*et al.*, 2005; Shirwaikar*et al.*, 2003). Indian review on plants secondary metabolites with anticancer activity, was allowed Vaidehi*et al*. (2017) to summarize 26 compounds isolated from *Gmelina arborea*, *Phyla nodiflora*, *Petreavolubilis*, *Clerodendrumphlomidis* and*Clerodendroninfortunatum*. However, there is a little data concerning Burkina Faso Verbenaceae species, specially on their chromatographic profile and their antioxidants activities. The present study will be focused on polyphenols profiles investigation of these species of Verbenaceae using 3 types of extracts and fractions directed against 3 antioxidant methods. Results analysis were combined with principal component analysis technic, cluster and Spearman correlation matrix analysis.

Material and Methods

Plant material

Aerial parts, stem leaves or leaves of *Duranta erecta* (fruits), *Duranta erecta* (leaves), *Gmelina arborea* (leaves), *Lantana cámara* (stem-leave) *Stachytarpheta angustifolia* (whole plant) and *Tectona grandis* (florals part), *Tectona grandis* (leaves), were collected in Burkina Faso between May and October 2013 at Gampella (25 Km East from Ouagadougou) and Komadougou (11 Km North of FadaN'Gourma), respectively. These plants were botanically identified by Professor Millogo-Rasolodimby from the Plant Biology Department of the University of Ouagadougou. The Voucher specimens were deposited in the OUA herbarium of the CIB (Centre d'Information sur la Biodiversité), UFR/SVT of the University of Ouagadougou.

Reagents and standards

Acetonitrile (HPLC grade), water (HPLC grade), ethanol (HPLC grade), ethyl acetate (analytical grade), hexane (analytical grade), sulfuric acid (analytical grade), and sodium phosphate were purchased from J. T. Baker (Xalostoc, Mexico). 2,2-Diphenyl-1-picrylhydrazyl (DPPH*), aluminum chloride, ammonium molybdate, and the references: quercetin, quercitrin (quercetin-3-rhamnoside), caffeic acid, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis Missouri, USA). The standards kaempferol-3,7-*O*-diglucoside, quercetin-3-*O*-[rhamnosyl-(1-6)-galactoside], kaempferol-3-*O*-[rhamnosyl-(1-6)-glucoside] came from Apin Chemicals Limited (Abingdon, Oxon, UK). Trichloroacetic acid and ferric chloride were obtained from Merck (Darmstadt, Germany). Potassium ferricyanide was purchased from Fermont (Monterry, Mexico).

Preparation of extracts

Polyphenols and flavonoids were extracted from dry ground (florals part, fruits, leaves, stem-leave or whole plant) (4 g) by maceration in 40 mL of: (1) ethanol 80% (v/v) and the mark re-extract with ethanol 20% (v/v) = ethanol extract, (2) acetone 100% and (3) decoction, for 24 hours in darkness, and at room temperature. The extracts were centrifuged (5000 rpm) for 10 min, at room temperature, and the supernatants separated. The pellets (only for EtOH-H_2O extract) were re-extracted in 100 mL of 20% ethanol (v/v) for 3 hours, centrifuged under the same conditions, and the supernatant decanted. Both supernatants were combined to form the total extracts, each extract was used for further analysis. Each totals extract was concentrated to dry for polyphenolic, flavonoids and antioxidants activities. Before an aliquot of EtOH-H_2O extract was concentrated to half the volume and then fractioned twice respectively with n-hexane, n-butanol and ethyl acetate solvents. The two organic fractions (ethyl acetate and hexane), the aqueous

fractions, and the separated aliquots of the total extracts, were individually concentrated under vacuum to dryness and then re-dissolved in 5 mL ethanol, aliquots were taken to be used in the determination of phenol, flavonoid and atomic absorption contents, in the HPLC-DAD analysis. All sampleswere directed against antioxidant activities.

Determination of phenolic content (PT)

Folin-Ciocalteu method was used for measurement of total content of phenolic compounds according to Nurmi *et al*. (1996) method, by linear regression analysis from the standard curve of gallic acid (Y = 0.003x+0.016; R^2 = 0.997). A 250 µl extract was mixed with 2.5 ml of desiionized water. Afterwards, 125 µL Folin-Ciocalteu reagent was added and the mixture could stand for 5 min. Finally, 375 µL of 20% Na_2CO3 was added. After 2 hours incubation at room temperature, the absorbance was measured at 760 nm on a Spectroscopic Analysis Mecasys (Optizen). Three replicates of each sample were analyzed.

Determination of flavonoid content (FT)

Flavonoid content was determined according to Lauranson-Broyer and Lebreton (1993) by linear regression analysis from the following standard curve of quercetin: Abs_{425nm} =0.025x + 0.014 [Quercetin], correlation coefficient R^2 = 0.998. The curve was registered after the addition of 60 µL of a freshly prepared 5% (w/v) aluminum chloride solution to 1 mL of quercetin solution (four different concentrations in the range of 100 to 1400 µg/mL). The absorbance was immediately registered after the addition of aluminum chloride, at 425 nm, using a SpectronicGenesys 2 espectrophotometer (Rochester, New York, USA). The flavonoid content in each sample was also registered after the addition of aluminum chloride and expressed as µg of quercetin equivalents/ g dry extract. The addition of aluminum chloride produces bathochromic shifts (which can be perceived by a yellow coloration) in flavonoids containing orthodihydroxyl groups, due to the formation of complexes between the aluminium and C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols ; complexes also are formed between the aluminum and the orthodihydroxyl groups in A or B-ring of flavonoids (Mabry *et al.*, 1970). The addition of aluminium chloride represents a standard procedure for reproducibility (Lauranson-Broyer and Lebreton, 1993). The determination of flavonoid content was estimated individually for three pools of samples.

HPLC-DAD Analysis

To determine the flavonoid profiles, aliquots of each extract (concentrated to dryness and re-dissolved in 5 mL ethanol, as mentioned in the section Preparation of extracts) were analyzed as previously described by Campos and Markham (2007) on a Perkin Elmer Series 200 HPLC system (Shelton, Connecticut, USA) and a Perkin Elmer Brownlee Analytical C18 column (4.6 x 250 mm, 5µm) (Shelton, Connecticut, USA), by an acidified acetonitrile-water gradient. Water adjusted to pH 2.5 with orthophosphoric acid was the solvent A, and acetonitrile was the solvent B, mixed according to the following gradient; starting with 100% A, decreasing to 91% over the next 12 mn, to 87% over next 8 mn, to 67% over the next 12 mn, to 57% over the next 10 mn, and held at this level until the end of the 60 mn analysis. Standard chromatograms were plotted at 260 and 340 nm. Spectral data for all peaks were accumulated in the range 220-400 nm using diode array detection (Perkin Elmer Series 200). Structural identification was obtained by direct comparisons of retention times and UV spectra of resolved compounds with those of standard; two kaempferol glycosides, identified as kaempferol-3,7-O-diglucoside and kaempferol-3-O-[rhamnosyl-(1-6)-glucoside], and one quercetin glycoside, identified as quercetin-3-O-[rhamnosyl-(1-6)-galactoside]. The structural information of compounds, for which standards were not availabe, was obtained from their spectral parameters according to the compilations of (Campos and Markham, 2007; Mabry *et al*., 1970). Quantitative determination was made by an external standard method, with the commercial reference (quercetrin), by area measurements, using the following standard curve: Area = -0.0046x + 0.0278 [Quercetrin], correlation coefficient r = 0.992. The content of each compound was expressed as µg of quercetrin equivalents/g G dry extract. The HPLC-DAD profiles were individually obtained and analyzed for the extracts from three pools of samples.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) of each sample was evaluated through the method developed by (Prieto *et al*., 1999), in which the reduction of Mo (VI) to Mo (V) is carried out by the antioxidant, forming a green phosphate/Mo (V) complex at acidic pH. Aliquots (100 µL) of each sample (containing 100 $\mu g.mL^{-1}$ of flavonols, respective concentrations of flavonols calculated from the standard curve of quercetin) were prepared and combined with 1 mL of a solution constituted of sulfuric acid (0.6 M), sodium phosphate (28 mM), ammonium molybdate

(4mM) and incubated at 95°C for 90 min. After reaching room temperature, the absorbance of each samples was registered at 695 nm against a blank prepared as indicated for the samples but adding ethanol instead of the sample. The reference quercetin was analyzed in the same manner. TAC was expressed as mg ascorbic acid equivalents. Ascorbic acid curve: A_{695} = 3,678x - 0,092[ascorbic acid], correlation coefficient R^2 = 0,998, constructed with ascorbic acid between 1.0 and 30.0 mg.mL^{-1}. The analysis was done for independence aliquots of the samples from three pools of samples.

Free radical scavenging activity (DPPH*)

The DPPH* method reported by Campos *et al*. (1994) was used to evaluate the free radical scavenging activity. Four to five flavonoid concentrations of each sample were individually added to a DPPH* solution (40 µg.mL^{-1} in ethanol) in such a way so as to maintain a final volume of 1 mL. The decrease in absorbance was determined at 523 nm after 10 min. The DPPH* concentrations of samples were plotted to determine by linear regression, the efficient concentration at 50 %, defined as the amount of antioxidant needed to decrease by 50 % the initial DPPH* concentration (EC_{50}). The following calibration curve, made with DPPH* between 6.25 and 100 µg.mL^{-1}, was used to calculate the DPPH* concentration (µg.mL^{-1}) in the reaction medium: A_{523} = 0,030x + 0,001 [DPPH*], correlation coefficient R^2 = 0,999. Antioxidant activities were expressed in terms of EC_{50} in µg.mL^{-1}. The analysis was separately done for the samples from three pools samples.

Iron Reducing Power (IRP)

The iron reducing power (IRP) method reported by Yang *et al*. (2008) was used to evaluate the iron reducing power of each simple. Aliquots (1 mL) of each sample were combined with 2.5 mL (phosphate buffer, 0.2 M, pH 6.6), 2.5 mL (potassium ferricyanide, 30mM) and incubated at 50 °C for 20 min. After, 2.5 mL trichloroacetic acid (0.6 M) was added and the mixture was centrifuged (2000 rpm for 10 min). From the upper layer, 2.5 mL of solution was removed and distilled water (2.5 mL) and ferric chloride (0.5 mL, 6 mM) were added to it. The absorbance at 700 nm of the formation of ferrous ions (Fe^{2+}) was registered after 10 min. The highest absorbance values indicated the greatest capacity of reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. Four flavonol concentrations (10-400 µL combined with the proper volumen of ethanol to reach 1 mL as final volumen) of each sample (respective concentrations of flavonols calculated from standard curve of quercetin) were evaluated. The reducing power was expressed in terms of EC_{50} (mg.mL^{-1}). The evaluation was separately done for the samples from three pools of samples.

Statistical Analysis

All assays were carried out in triplicates and results are expressed as statistical comparisons it was done with the PAST v2.14 (Hammer *et al*., 2001), using Spearman correlation. Differences were considered to be significant at p <0.05.

Results and Discussion

Phytochemical investigation

Plant differentiation analysis

The chemical characterization of the 5 Verbenaceae species studied in this work, was carried out through the total phenols, total flavonoids and HPLC-DAD analysis. Different technics were used to appreciate secondary metabolites differentiation in these species such as principal component analysis (PCA) factors, cluster analysis and spearman correlation matrix.

Two well-defined main groups were formed according the analysis of the fractions extracts (Figure 1), where group 1 is composed of SAN-FH (*S. angustifolia)*, GAR-FH (*G. arborea*), GAR-FB (*G. arborea*), GAR-FAE (*G. arborea*), SAN-Faq (*S. angustifolia),* GAR-Faq (*G. arborea*); and the second group LAC-FB (*L. camara)*, LAC-FH (*L. camara)*, LAC-FAE (*L. cámara)*, SAN-FAE (*S. angustifolia)*, LAC-Faq (*L camara)* and SAN-FB (*S. angustifolia)*. Within this group it can be observed that LAC-FB is completely isolated. This indication is corroborated by a principal component analysis (PCA) (Figure 2); a Spearman correlation was also carried out where the correlations are shown for which variables are significant for a p <0.05 (Table 1).

For the analysis of the decoction extract, two well-defined main groups were formed (Figure 3), where the first group is formed specifically by *L. camara*1 and *G. arborea*1; and the second group consists of: *S. angustifolia*1, *T. grandis* fruit1, *D. erecta* fruit1, *D. erecta* leaves1 and *T. grandis* leaves1. In this second group, there is a subgroup in which we observe that both the leaves and fruits are grouped. To check up these results, principal component analysis (PCA) was carried out (Figure 4); likewise, a Spearman correlation was made (Table 2). The significant correlations were revealed for the analysis of the decoction extract. Its was observing that for this type of analysis the correlation shown is negative and significant for PT vs TAC and FT vs DPPH*.

EtOH-H_2O extraction analysis show two well-defined main groups formed (Figure 5). Group 1 constituted of *T. grandis* leaves2, *L. camara*2 and *S.*

*angustifolia*2; and the second group including of: *D. erecta* fruit2, *T. grandis* fruit2, *D. erecta* leaves2 and *G. arborea*2. The subgroup constituted of fruit and leaves was conserved like in the previous analysis (Figure 3). Also, the difference was observed between leaves (*D. erecta, T. grandis*), Stem-leave (*L. camara*), whole plant (*S. angustifolia*) and fruits (*D. erecta, T. grandis*). To be able to know if in a certain moment this analysis is more discriminatory in function of a separation for each part of the plant, a principal component analysis (PCA) was carried out (Figure 6). Likewise, a Spearman correlation was made (Table 3). Then, significant correlations for these results analysis are observed.

Acetonic extraction analysis allowed us to highlight two well-defined main groups formed (Figure 7). First group only contains *D. erecta* fruit3, while the second group is composed of *T. grandis* fruit 3, *T. grandis* leaves3, *L. camara*3, *D. erecta* leaves3, *S. angustifolia*3 and *G. arborea*3. In turn, this group is made up of two subgroups, which allow us to see a specific group for *T. grandis* (fruit and leaves), which allows to observe a discrimination for this Verbenaceae. Figure 7 clearly shows the unicity of *D. erecta* in the group, which, in general its due to the amount of its secondary metabolites content. Also, principal component analysis (Figure 8) allowed to observe how this variety (*D. erecta*) is practically isolated. Spearman correlation (Table 4) allowed to observe certain significant values ($p < 0.05$), but only two were observed that are PT vs DPPH * (negative correlation) and TAC vs IRP.

Phenol and flavonoids contents

The total phenol was obtained using three different extraction methods. The results were ranged in the Table 5, 6, 7. Its varied between 111.26 ± 10.96 to 1.79 ± 0.12 mg GAE/g dry tissue. Among these 3 types of total extracts, EtOH-H_2O, given the bests contents ranged between 111.26 ± 10.96 to 19.48 ± 1.68 mg GAE/g dry tissue. Follow of that of acetone extract 24.85 ± 1.52 to 1.79 ± 012 mg GAE/g dry tissue and lowest content is detained with decoction extract (19.07 ± 1.89 to 5.53 ± 0.23 mg GAE/g dry tissue). In term of total flavonoids content, the results were ranged between 4.42 ± 0.69 to 0.12 ± 0.03 mg QE/g dry tissue. Excepted EtOH-H_2O total extract content, acetone and decoction are both similar contents (Table 5, 6, 7).

According to literature investigation, bark aqueous total extract of *Gmelina arborea*Roxb. was study by Attanayake*et al*., (2015) using the same procedure. These authors found 13.00 mg GAE.g^{-1} dry tissue for total phenol and 1.77 ± 0.1 QE/g of dry weight for total flavonoid contents. Comparing to decoction extract, their results as better than us respectively with total phenol (10.91 ± 0.21 mg GAE/g dry tissue) and total flavonoids (0.41 ± 0.03 mg QE/g dry tissue). These results seem to be logical by taking account of the origin and the part of the plant used. Other researchers (Patil *et al*., 2009) which evaluated methanolic extract of stem-bark of *G. arborea*found 356 mgGAE.g^{-1} dry tissue. Alcoholic extracts of *G. arborea* was reported to exhibit anti-inflammatory and wound healing properties (Dhakulkar*et al*., 2005; Shirwaikar*et al*., 2003) and crude drug extract are also known to inhibit platelet aggregation (Dhakulkar*et al*., 2005; Faiza &Darakhshanda, 1998).

HPLC-DAD analysis

56 polyphenolic compounds were detected in different parts of Verbenaceae plant (leaves, fruits, stem-leaves and whole plant), using high performance liquid chromatography with photodiode matrix. The most represented compounds were Luteolin (7), Chlorogenic acid (5), Genkwanin (4), Rosmarinic acid (4), and Scutellarein-6,4'-dimethylether (4). Table 8 contains all detail concerning nature of sample, retention time and the wavelengh. UV spectra and their retention time are also presented in the Figure 9a-l, Figure 10a-l, Figure 11a-l, Figure 12a-l, Figure 13a-h.

Greenham*et al*. (2003) also highlighted apigenin, genkwanin, luteolin, scutellarein and their derivatives in various *Ocimum* species. Generaly, phenolic acids were the most abundant compound in the aerial tissues of both taxa here analyzed. These phenols play important roles in plants such as protection against herbivores and pathogens, cementing material joining phenolic polymers to polysaccharides of cell walls (Bangou *et al*., 2012a; Wallace and Fry, 1994). Regulation of cell growth and division (Binns*et al*., 1987), and inhibition of ion intake by a generalized increase in membrane permeability to inorganic ions as a mode of action of allelopathic compounds (Glass, 1974). They also have relevant antioxidant properties (Ávila-Reyes *et al.*, 2010; Almaraz-Abarca*et al*., 2007; Rice-Evans *et al*., 1997).

Variation in the foliar phenol profiles

The foliar phenol profiles have been reported to have a species-specific tendency in several groups of plants like in Agave (Almaraz-Abarca*et al*., 2013). This tendency was corroborated in study for *P. vulgaris* and *P. coccineus* Reyes-Martínez *et al*. (2014). Cluster analysis (Figure 14) show that there is some chemical difference in the foliar phenol composition. This analysis is based on a binary matrix of presence–absence constructed with the

foliar phenol profile of each variety. Each variety had a typical foliar phenol profile; this makes those profiles important tools for the evaluation of variety authenticity for this crop plant. In this case, two well-defined groups were delimited which observing in groups A and B the varieties of *L camara* (ethanol-water) and *S. angustifolia* (acetone) respectively, were separated from the rest (Figure 14). This difference indicates the variability of phenolic compounds. According to Millogo (2008) plants polyphenolic compound contents, would be related to the climatic and edaphic conditions (Millogo, 2008). Thus, these contents would vary according to the zones, at the richness of the basement and the precipitation. That would explain partly the presence or not of certain secondary metabolites in a plant, and in particular the differences of their contents. Thus, a plant can have beneficial properties here, while the same specie would be toxic at 100 km for example. This variability of plants species makes possible, the acceptance of traditional medicine data observed throughout the world. With the climatic changes increased in this last decade, which would be the value of these secondary metabolites in the next years?

Biological investigation

Antioxidants capabilities

Radical DPPH* assay was used to evaluate the antioxidant activity of 5 species of Verbenaceae. Its well-known that antiradical activity of sample was expressed as EC_{50} value, which represented the effective concentration of sample required to scavenge 50% of radical's DPPH. Results were varied between 31.07 ± 0.02 (*D. erecta* fruit) to 1.43 ± 0.02 (*L. camara*) ug/mL. According to the radical DPPH principle, the bests results will be the extracts which gave the low value. Then, the best and the lowest values were obtained by acetone total extract with 1.43 ± 0.02 and 31.07 ± 0.02 µg/mL, respectively (Table 5, 6, 7). Decoction and EtOH-H_2O extracts seem to be poor in radical scavenging activity, comparatively.

What about total antioxidant capacity? The results were ranged between 10.28 ± 0.07 (*D. erecta* leaves) to 0.69 ± 0.01 (*L. camara*) µg AA/mL. EtOH-H_2O extract given the bests values ranging between 10.28 ± 0.07 to 6.36 ± 0.12 µg AA/mL, follow of that of acetone extract where the best value was 2.24 ± 0.03 µg AA/mL (Table 5, 6, 7). Decoction extract contains lowest values.

The iron reducing power (IRP) method reported by Yang, Guo and Yuan (2008) was used to evaluate the iron reducing power of each simple. The highest absorbance values indicated the greatest capacity of reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. The results of this experimentation were ranged between 0.80 ± 0.01 to 0.035 ± 0.00 µg/mL. In the descending order, the bests results were EtOH-H_2O > acetone and decoction extracts. The best result is obtained with *D. erecta* (leaves) and the lowest *D. erecta* (fruits).

It comes out from this study that *Duranta erecta* (fruits) have the lowest value antioxidant activity with the 3 types of extracts. Specie which given the best anti-DPPH* activity was *L. camara* with 1.43 ± 0.02 µg/mL. This activity was obtained with acetone extract.

All plants were seemed to have same activity of total antioxidant capacity with EtOH-H_2O extract. Among these species *Stachytarphetaangustifolia* presented the best activity with the 3 types of extracts. Former investigation made by Awah*et al*. (2010) seem to collaborate with our study. In effect, these researchers used EtOH extract (80%) of *Stachytarpheta angustifolia* and showed that it was significantly inhibited free radicals, lipid peroxidation in vitro and decreased the phenotypic expression of CD38 and CD69 (Awah*et al*., 2010).

Methanolic extract of *D. erecta,L. camara* and *S.angustifolia* belonging to the Verbenaceae family was already showed (Bangou *et al*., 2012b) interesting activities on radical-DPPH* and Ion (III) to iron (II)-reducing activity (FRAP).Crude extracts of *Vitex agnus-castus* was showed to have potent antioxidant activity (Sultan and Çelik, 2013).Indian researchers were showed that oleanolic acid, clerodinin A and flavonoidal compounds of Verbenaceae species comers from *C. infortunatum*and *Gmelina arborea* as antioxidant and anticancer agents, respectively (Vaidehi*et al*., 2017).

Although the quantity differs from quality, it's well-know that luteolin is a natural flavonoids which has a potential antioxidant activity, an anti-inflammatory activity, induces apotosis and is chemopreventive in cancer treatments (Chen *et al*., 2018; Kang *et al*., 2017; Cai *et al*., 2007; Rice-Evans *et al*., 1996; Lee *et al*., 1995). Genkwanin and Scutellarein-6,4'-dimethyl ether are considered like an unglycosylated flavonoids with anti-inflammatory properties and antioxidant (Gao *et al*., 2014; Narain*et al*., 1976). Chlorogenic acid and androsmarinic acid are considered associated with the regulation of glucose and lipid metabolism, as well as a cancer inhibitor, analgesic, antipyretic, platelet inhibitory, antioxidant and anti-inflammatory properties (Adomako-Bonsu *et al*., 2017; Santana-Gálvez*et al*., 2017; Tajik *et al*.

2017; Meng *et al*., 2013; Fuentes *et al*., 2004; Baba*et al*., 2004; Petersen *et al*., 2003).

Conclusion

This study showed that the varieties of Verbenaceae can synthesizes, accumulate and classify in function of its secondary metabolites. That was facilitated using the method of principal component analysis. 56 polyphenolics compounds were characterized in these plants species including approximately 40 flavonoids and their derivatives detected. Flavonols were most represented with luteolin and derivatives. The bests results were obtained in the descending with EtOH-H_2O > acetone and decoction extracts. *D. erecta*,*L. camara* and *S.angustifolia* were showed interesting activities on radical-DPPH* anti-TAC and Ion (III) to iron (II)-reducing activity (IRP). Our next studies on these plants species will aim at making a bio-guided evaluation to (1) identify the polyphenolic compounds implied in the antioxidants activities *in vivo*, (2) to check literatures informations such as anti-inflammatory and cyto-toxicity properties.

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Table 1. Spearman correlation matrix of attributes in the study area : PT (Phenolic Content), FT (Flavonoid Content), TAC (Total Antioxidant Capacity), FR (Iron Reducing Power) and DPPH* (Free Radical Scavenging Activity)

Variable	PT	FT	TAC	IRP	DPPH*
FT	0.56*				
TAC	-0.01ns	0.62**			
FR	0.64**	0.74ns	0.42ns		
DPPH*	-0.04ns	0.60**	0.97***	0.39ns	
Note: Asterisks indicate significance: *= at $p< 0.1$, ** at $p<0.05$ and *** at $p< 0.001$; ns = not significant					

Table 2. Spearman correlation matrix to decoction extraction : PT (Phenolic Content), FT (Flavonoids Content), TAC (Total Antioxidant Capacity), FR (Iron Reducing Power) and DPPH* (Free Radical Scavenging Activity)

Variable	PT	FT	TAC	IRP	DPPH*	
FT	0.60ns					
TAC	-0.71*	-0.46ns				
FR	0.03ns	-0.071ns	0.10ns			
DPPH*	-0.50ns	-0.85**	0.50ns	0.46ns		
Note: Asterisks indicate significance: *= at $p< 0.1$, ** at $p<0.05$ and *** at $p< 0.001$; ns = not significant						

Table 3. Spearman correlation matrix to EtOH-H_2O extract: PT (Phenolic Content), FT (Flavonoid Content), TAC (Total Antioxidant Capacity), FR (Iron Reducing Power) and DPPH* (Free Radical Scavenging Activity)

Variable	PT	FT	TAC	IRP	DPPH*	
FT	0.75*					
TAC	0.92***	0.64ns				
FR	0.96***	0.71*	0.96***			
DPPH*	0.53ns	0.10ns	0.53ns	0.60ns		
Note: Asterisks indicate significance: *= at p< 0.1, ** at $p<0.05$ and *** at $p< 0.001$; ns = not significant						

Table 4. Spearman correlation matrix to acetone extract: PT (Phenolic Content), FT (Flavonoid Content), TAC (Total Antioxidant Capacity), FR (Iron Reducing Power) and DPPH* (Free Radical Scavenging Activity)

Variable	PT	FT	TAC	IRP	DPPH*	
FT	0.14ns					
TAC	-0.14ns	-0.28ns				
FR	-0.53ns	-0.21ns	0.67*			
DPPH*	-0.75*	-0.53ns	0.03ns	0.46ns		
Note: Asterisks indicate significance: *= at $p< 0.1$, ** at $p<0.05$ and *** at $p< 0.001$; ns = not						

significant

Table 5. Total phenols, total flavonoid content, free radical scavenging activity (EC_{50}), TAC and iron-reducing power of the foliar extracts of 7 varieties of Verbenaceae, obtained by EtOH-H_2O extract

Samples	Total phenols (mg GAE/ g^{-1} dry tissue)	Total Flavonoids (mg QE/g^{-1} dry tissue)	DPPH* EC_{50} (µg mL^{-1})	TAC (µg AA mL^{-1})	IRP (µg mL^{-1})
G. arborea	111.255 ± 10.961 a	4.116 ± 0.689 a	9.0346 ± 0.222 e	9.996 ± 0.329 a	0.744 ± 0.029 b
D. erecta leaves	72.700 ± 8.838 b	2.583 ± 0.175 bcd	10.590 ± 0.110 c	10.280 ± 0.072 a	0.796 ± 0.010 a
T. grandis leaves	68.144 ± 4.670 bc	3.216 ± 0.500 abc	9.262 ± 0.198 d	8.073 ± 0.119 c	0.610 ± 0.006 c
D. erecta fruit	66.033 ± 3.179 bc	1.583 ± 0.189 de	22.142 ± 1.211 a	8.864 ± 0.205 b	0.526 ± 0.009 d
T. grandis fruit	55.700 ± 1.763 c	2.016 ± 0.480 de	17.921 ± 0.282 b	7.496 ± 0.168 d	0.335 ± 0.014 e
S. angustifolia	30.811 ± 1.575 d	1.383 ± 0.189 e	10.277 ± 0.213 cd	8.126 ± 0.246 c	0.296 ± 0.009 f
L. camara	27.144 ± 2.589 d	2.383 ± 0.305 cde	6.814 ± 0.137 f	6.810 ± 0.235 e	0.237 ± 0.007 g

Notes: GAE, gallic acid equivalents; QE, quercetin equivalents; EC_{50}, efficient concentration at 50%; TAC, total antioxidant capacity; RP, iron-reducing power.
The values represent the mean and standard deviation of three independent analyses.
Different letters in the same column mean significant differences (Duncan's multiple range test; $p< 0.5$).

Table 6. Total phenols, total flavonoid content, free radical scavenging activity (EC_{50}), TAC and iron-reducing power of the foliar extracts of 7 varieties of Verbenaceae, obtained by acetone extract

Samples	Total phenols (mg GAE/ g^{-1} dry tissue)	Total Flavonoids (mg QE/g^{-1}dry tissue)	DPPH* EC_{50} (µg mL^{-1})	TAC (µg AA mL^{-1})	IRP (µg mL^{-1})
T grandis leaves	24.058 ± 2.657 a	0.221 ± 0.034 f	3.401 ± 0.094 d	1.388 ± 0.062 c	0.1773 ± 0.0040 bc
T.grandis fruts	19.470 ± 1.301 b	0.630 ± 0.067 e	6.353 ± 1.152 b	0.975 ± 0.017 e	0.1389 ± 0.0024 d
L. camara	5.558 ± 0.976 cd	1.126 ± 0.118 bc	1.430 ± 0.024 f	1.159 ± 0.032 d	0.0556 ± 0.0004 g
G. arborea	3.636 ± 0.088 de	1.146 ± 0.040 a	3.135 ± 0.077 de	0.988 ± 0.022 e	0.1404 ± 0.0021 d
D. erecta leaves	3.270 ± 0.115 de	0.628 ± 0.045 e	6.774 ± 0.195 b	1.918 ± 0.034 b	0.2048 ± 0.0017 a
S. angustifolia	2.970 ± 0.480 de	0.805 ± 0.030 de	4.470 ± 0.073 c	2.237 ± 0.032 a	0.1582 ± 0.0023 cd
D.erecta frut	1.792 ± 0.117 e	0.131 ± 0.020 f	31.068 ± 0.191 a	1.040 ± 0.015 e	0.1559 ± 0.0321 cd

Notes: GAE, gallic acid equivalents; QE, quercetin equivalents; EC_{50}, efficient concentration at 50%; TAC, total antioxidant capacity; RP, iron-reducing power.
The values represent the mean and standard deviation of three independent analyses.
Different letters in the same column mean significant differences (Duncan's multiple range test; $p< 0.5$).

Table 7. Total phenols, total flavonoid content, free radical scavenging activity (EC_{50}), TAC and iron-reducing power of the foliar extracts of 7 varieties of Verbenaceae, obtained by decoction extract

Samples	Total phenols (mg GAE/ g^{-1} dry tissue)	Total Flavonoids (mg QE/g^{-1} dry tissue)	DPPH* EC_{50} (µg mL^{-1})	TAC (µg AA mL^{-1})	IRP (µg mL^{-1})
L. camara	19.073 ± 1.889 a	0.502 ± 0.015 b	4.7312 ± 0.1341 e	0.696 ± 0.014 e	0.050 ± 0.0005 cde
T. grandis leaves	12.170 ± 1.316 b	0.975 ± 0.027 a	5.8954 ± 0.0619 d	1.050 ± 0.014 c	0.1202 ± 0.0229 a
G. arborea	10.906 ± 0.213 bc	0.514 ± 0.038 b	3.5752 ± 0.0970 g	0.720 ± 0.035 e	0.0585 ± 0.0009 cd
D. erecta leaves	10.893 ± 0.583 bc	0.437 ± 0.048 b	6.4157 ± 0.2704 c	1.139 ± 0.070 b	0.0452 ± 0.0005 de
T. grandis fruit	8.143 ± 0.173 cd	0.416 ± 0.042 b	12.2176 ± 0.1687 a	0.753 ± 0.019 e	0.0959 ± 0.0035 b
D. erecta fruit	7.156 ± 0.354 cd	0.537 ± 0.081 b	3.8722 ± 0.0113 f	1.011 ± 0.007 cd	0.0346 ± 0.0008 e
S. angustifolia	5.531 ± 0.229 d	0.122 ± 0.034 c	10.175 ± 0.1304 b	1.856 ± 0.034 a	0.0750 ± 0.0035 b

Notes: GAE, gallic acid equivalents; QE, quercetin equivalents; EC_{50}, efficient concentration at 50%; TAC, total antioxidant capacity; RP, iron-reducing power.
The values represent the mean and standard deviation of three independent analyses.
Different letters in the same column mean significant differences (Duncan's multiple range test; $p< 0.5$).

Table 8. Results obtained by HPLC-DAD analysis

Sample (extract)	Compound	RT (min)	λ_{max}(nm)
L. camara **(acetone)**	**1.-** Derivative of apigenin-7-O-(rhamnosyl(1-2)glucoside	20.009	274, 336nm
	2.- Scutellarein-6,4´-dimethylether	22.4	274, 332nm
S. tarchytarpheta**(acetone)**	**3.-** Chlorogenic acid	22.121	245sh, 296sh, 327nm
	4.- Derivative rosmarinic acid	35.996	250sh, 290sh, 325nm
	5.- Derivative of scutellarein-6,4'-dimethylether	36.344	235sh, 282, 332nm
	6.- Luteolin	36.612	254, 267sh, 290sh, 349nm
	7.-Rosmarinic acid	37.712	248sh, 289sh, 325nm
	8.- Apigenic-7-o-(apiosyl(1-2)glucoside)	40.264	266, 335nm
	9.- Orientin	47.465	253, 266sh, 290sh, 347nm

	10.- Genkwanin	52.554	267, 291sh, 331nm
L. camara **(decoction)**	**11.-** Chlorogenic acid	22.4	245sh, 296sh, 323nm
	12.- P-coumaric acid	34.426	225, 293sh, 308nm
	13.- Derivative of luteolin-7,4'-di-O-glucuronide	37.374	273, 340nm
	14.- Scutellarein-6,4´-dimethylether	40.469	273, 331nm
S. tarchytarpheta**(decoction)**	**15.-** Luteolin	47.201	252, 267sh, 290sh, 346nm
	16.- Genkwanin	52.144	267, 290sh, 331nm
L. camara **(ethanol-water)**	**17.-** Chlorogenic acid	22.4	245sh, 296sh, 327nm
	18.- Genkwanin	34.837	267, 330nm
	19.-Scaposin	36.201	268, 331nm
	20.-Dof tricetin-3´,4´,5´-trimethylether	37.448	253sh, 275, 337nm
	21.-Dof acacetin-7-O-(rhamnosyl(1-2)glucoside	40.601	272, 330nm
	22.- 3-O-methylquercetin	62.865	255, 263sh, 293sh,354nm
Duranta erecta **(leaves)(aectone)**	**23.-** Chlorogenic acid	22.678	245sh, 326nm
	24.- Rosmarinic acid	23.573	249sh, 291sh, 322nm
	25.- Scutelarein-6,4´-dimethylether	41.378	276, 331nm
Duranta erecta **(fruits) (acetone)**	**26.-** P-coumaric acid	26.448	228sh, 293sh, 310nm
	27.- Chlorogenic acid	28.721	245sh, 296sh, 325nm

	28.- Rosmarinic acid	32.153	250sh, 290sh, 322nm
***Gmelina arborea*- (acetone)**	**29.-**Dof 7-,4´-di-dimethylflavonol	35.79	254, 266sh, 296sh, 343nm
	30.- 7-,4´-di-dimethylflavonol	36.406	252, 266sh, 345nm
***T. grandis* (floral parts)(acetone)**	**31.-**Cafeic acid	28.853	240sh, 296sh, 323nm
	32.- Luteolin	47.67	253, 267sh, 291sh 348nm
	33.- Apigenin	52.628	267, 290sh, 333nm
	34.-Chrysoetriol	53.654	242sh, 250, 267, 346nm
***Tectona grandis* (leaves) (acetone)**	**35.-**Rosmarinic acid	26.653	216sh, 250sh, 290sh, 320nm
	36.- Derivative of 7,4'-di-O-dimethylflavonol	35.79	255 267sh, 342nm
	37.- Derivative of luteolin	36.481	222sh, 253sh, 267sh, 345nm
	38.- Apigenin-7-O-glucoside	40.117	223sh, 267, 335nm
	39.- Vitexin	41.364	241, 267, 290sh, 336nm
	40.- Luteolin	47.201	224sh, 253sh, 266sh, 291sh, 346nm
***Duranta erecta* (leaves)(decoction)**	**41.-**Dof acacetin-7-O-(rhamnosyl(1-2)glucoside)	40.601	272, 331nm
	42.- Scutellarein-6,4´-dimethylether	41.364	274, 332nm
***Duranta erecta* (fruits) (decoction)**	n/d	----	----
***Gmelina arbórea* (decoction)**	**43.-**Dofsaponarin	29.85	269, 334nm
	44.-Dof 7,4´-di-O-dimethylflavonol	36.289	253, 266sh, 291sh, 338nm

	45.- Luteolin 3´-O-glucoside	41.481	240, 269, 289sh, 338nm
	46.- Luteolin	47.607	252, 267, 291sh, 347nm
	47.- Dof 8-hydroxy-apigenin-8-O-glucuronide	52.701	269, 294sh, 331nm
T. grandis* (floral parts) (*decoction)	**48.-** Luteolin	46.306	253, 267sh, 291sh, 349nm
	49.- Vitexin	51.865	266, 291sh, 335nm
	50.-Dof chrysoeriol-5-O-glucoside	53.097	240sh, 250sh, 265, 290sh, 344nm
***Tectona grandis* (leaves) (decoction)**	**51.-** Derivative of apigenin-7-O-[rhamnosyl(1-2)glucoside]	35.101	269, 290sh, 337nm
	52.-Dof luteolin-7-O-rutinoside	36.142	254, 267sh, 290sh, 343nm
	53.- Luteolin	36.406	253, 267sh, 290sh, 346nm
	54.-Dof chrysoeriol-6,8-di-glucoside	47.128	253sh, 267, 290sh, 346nm
	55.- Genkwanin	52.144	267, 290sh, 332nm
	56.-Dof tricin	53.376	247sh, 267sh, 292sh, 344nm

n/d: no determinated

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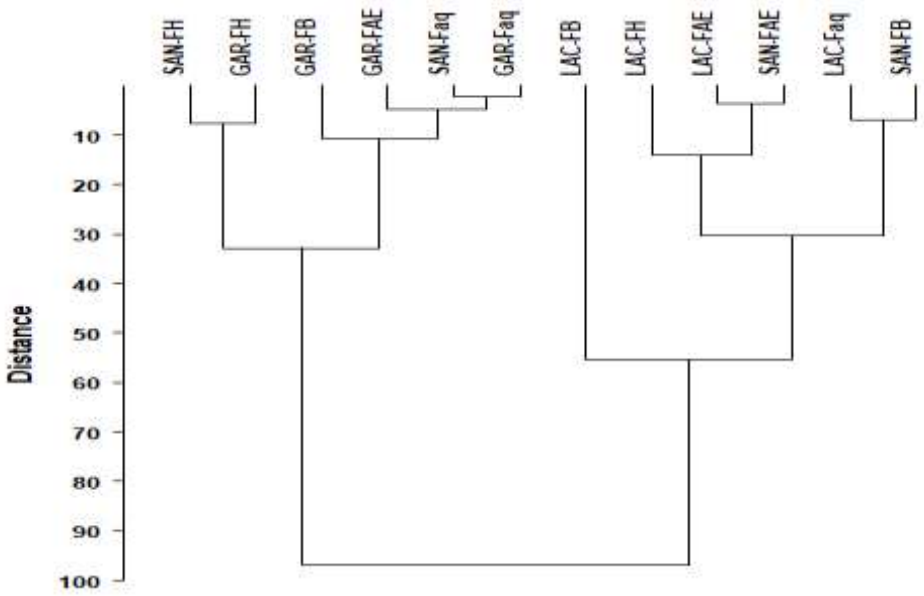

Fig. 1. Results of cluster analysis comparing foliar phenol profiles for seven varieties of Verbenaceae and derived fraction

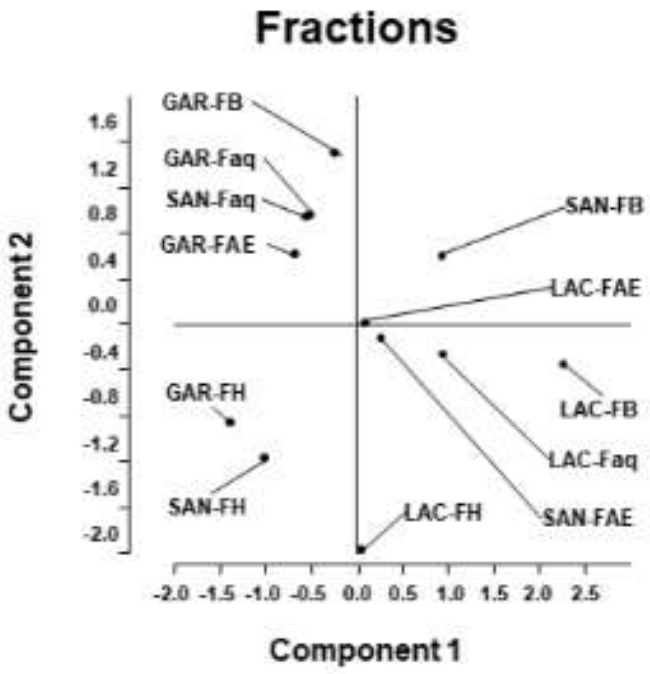

Fig. 2. Independent variables correlated with PCA factors F1 and F2.

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Decoction extract

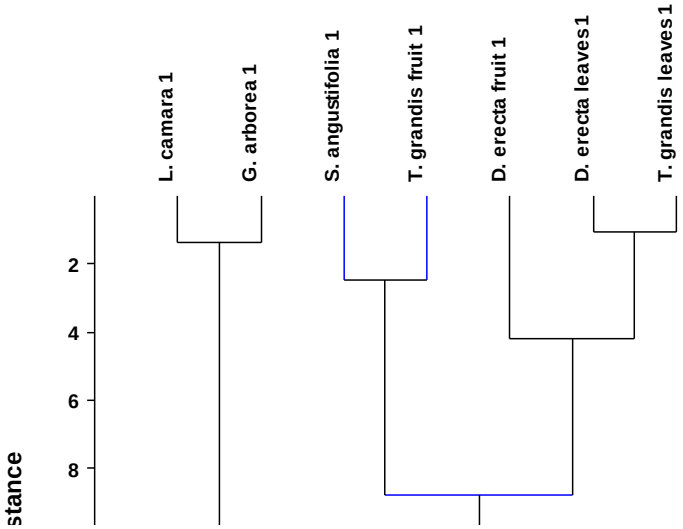

Fig. 3. Results of cluste

Decoction Acqueux

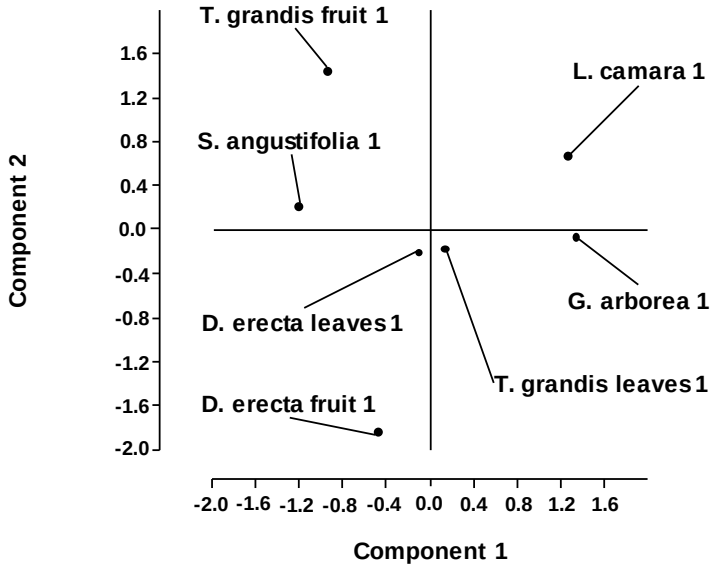

Fig. 4. Independe

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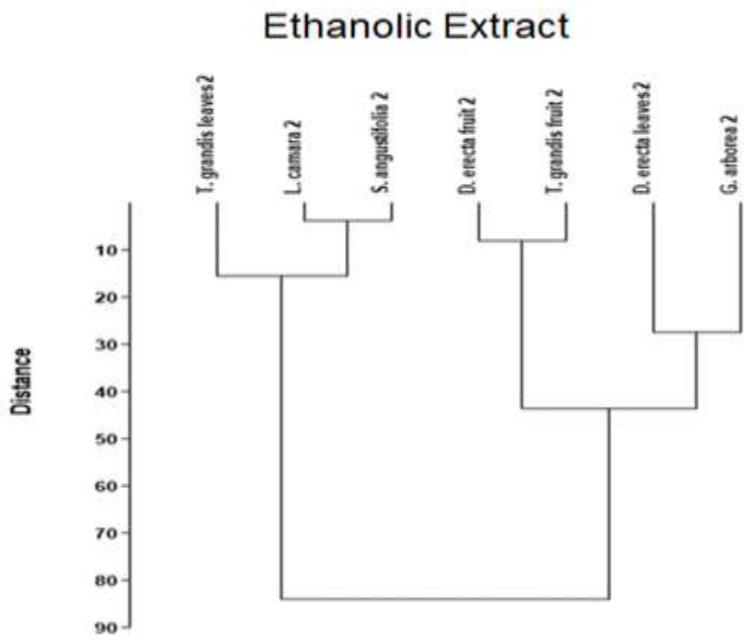

Fig. 5. Results of cluster analysis comparing foliar phenol profiles for seven varieties of Verbenaceae

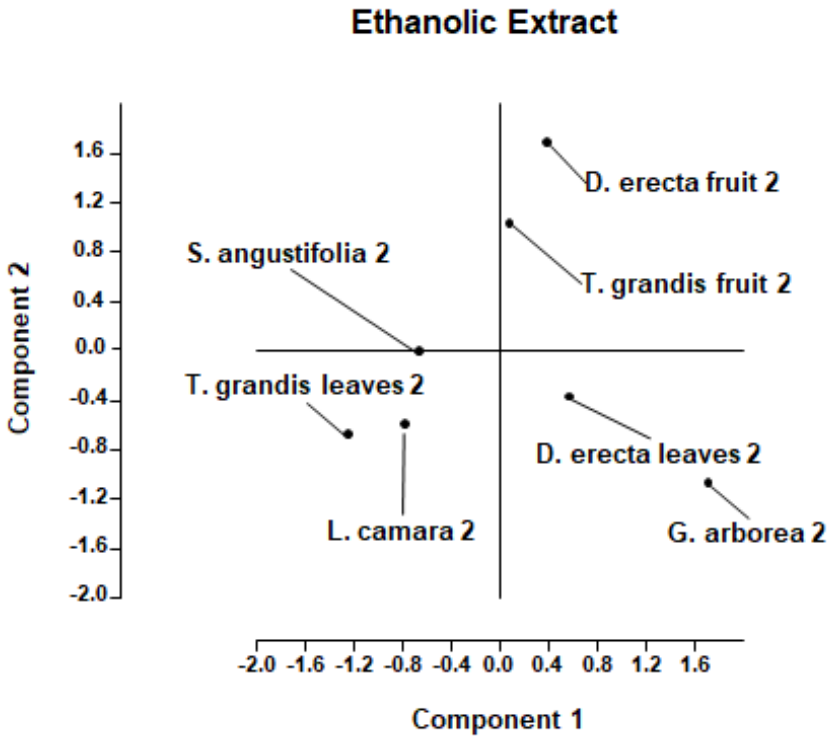

Fig. 6. Independent variables correlated with PCA factors F1 and F2.

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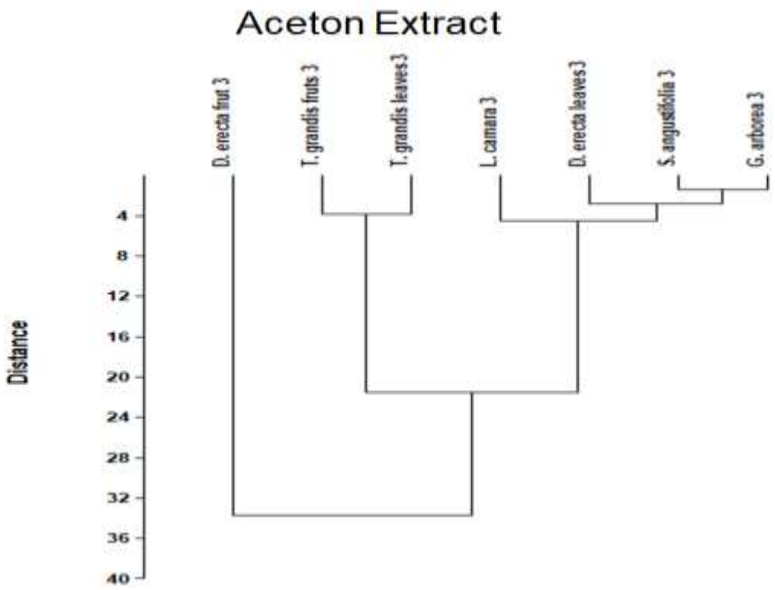

Fig. 7. Results of cluster analysis comparing foliar phenol profiles for seven varieties of Verbenaceae

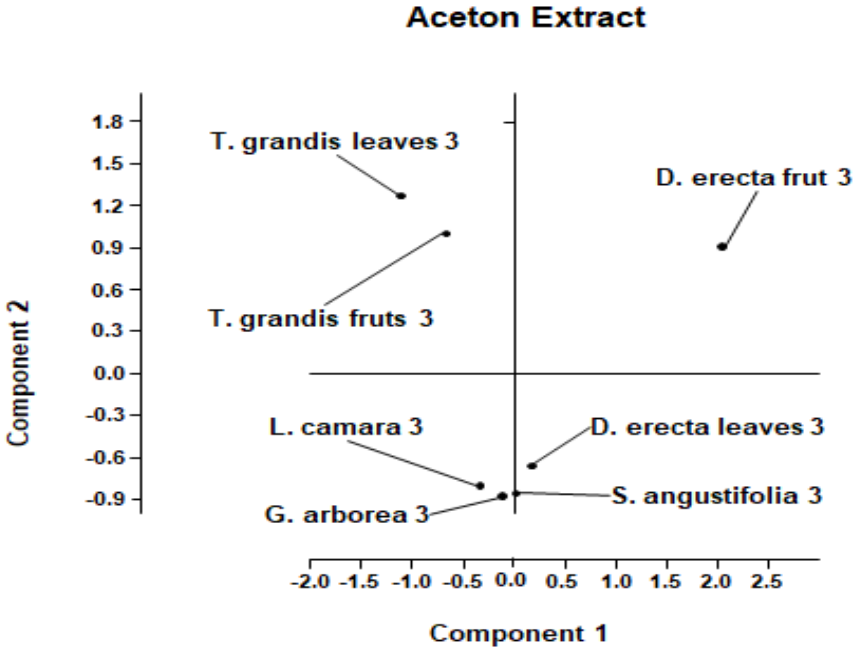

Fig. 8. Independent variables correlated with PCA factors F1 and F2.

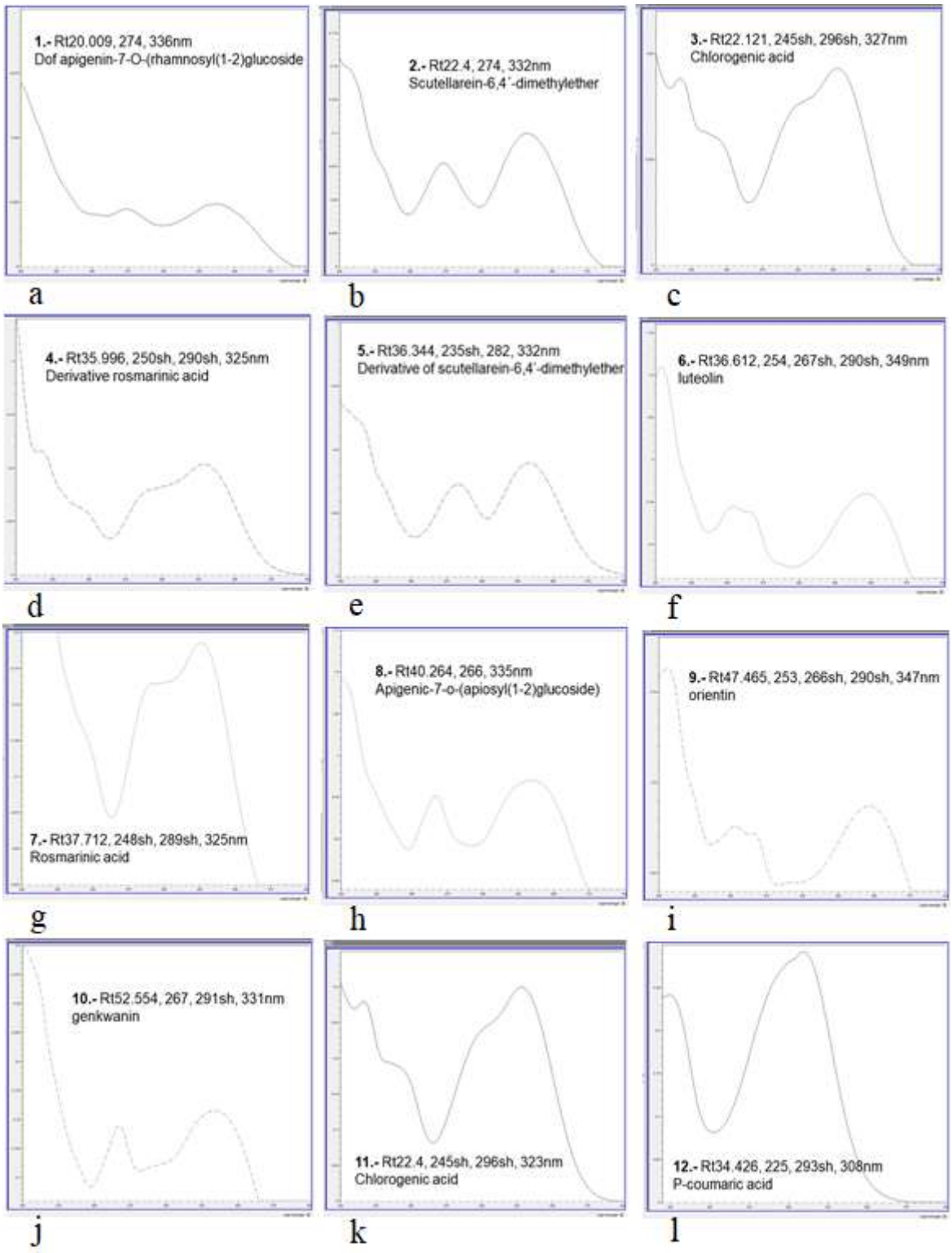

Figure 9a-l. UV spectra, λ_{max}, and retention time of the phenolic compounds 1 to 12of the foliar tissues

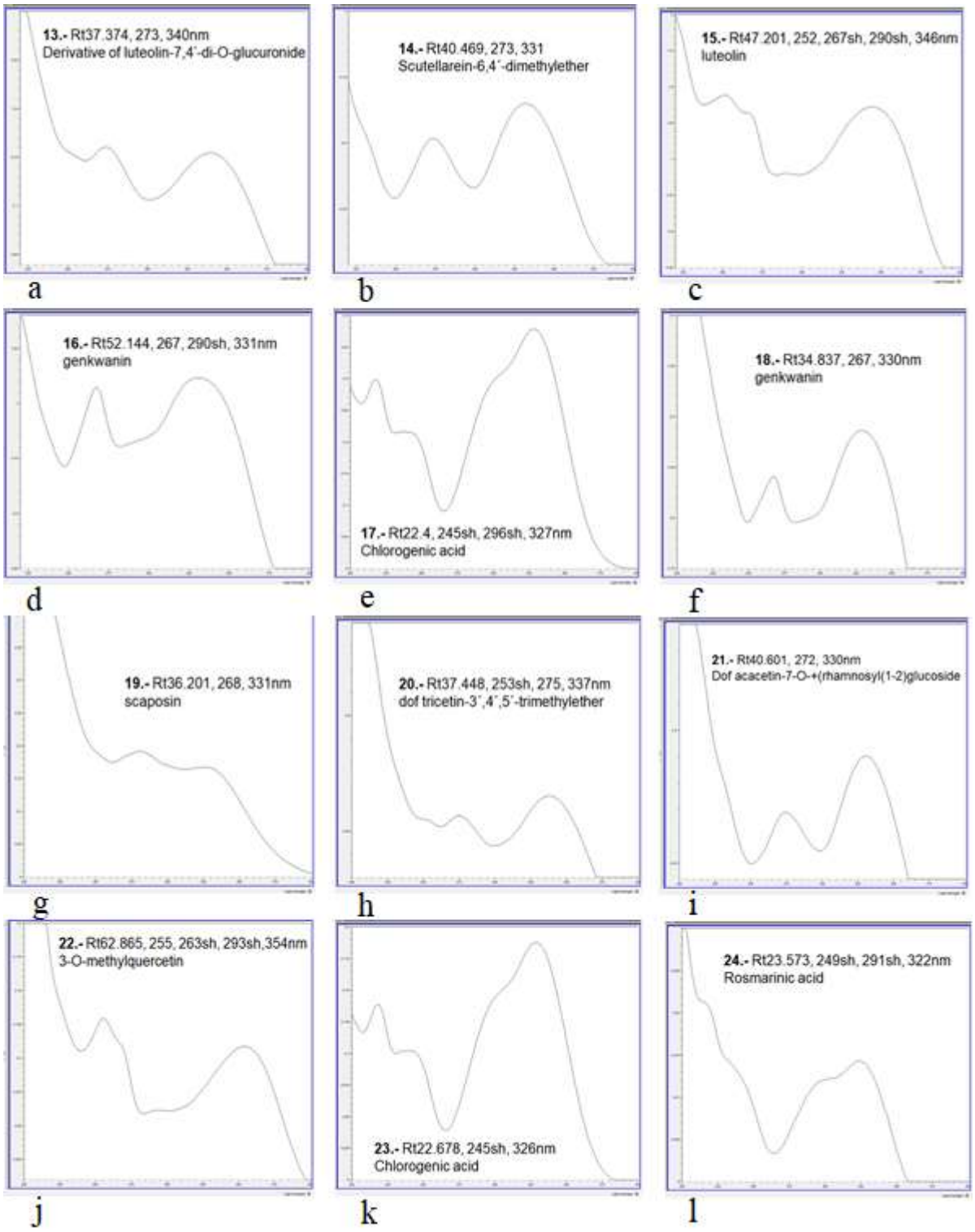

Figure 10a-l. UV spectra, λ_{max}, and retention time of the phenolic compounds 13 to 24of the foliar tissues

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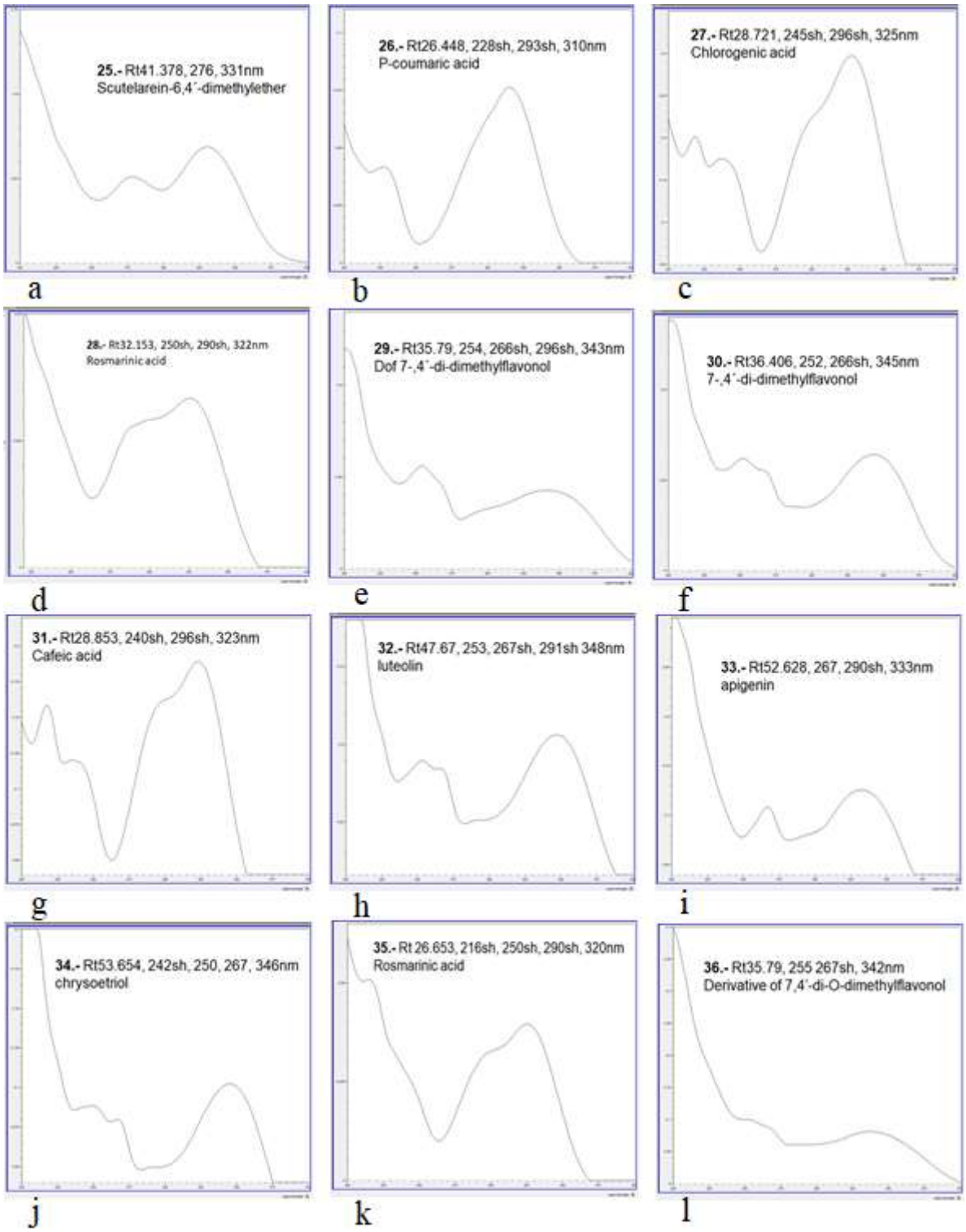

Figure 11a-l. UV spectra, λ_{max}, and retention time of the phenolic compounds 25 to 36of the foliar tissues

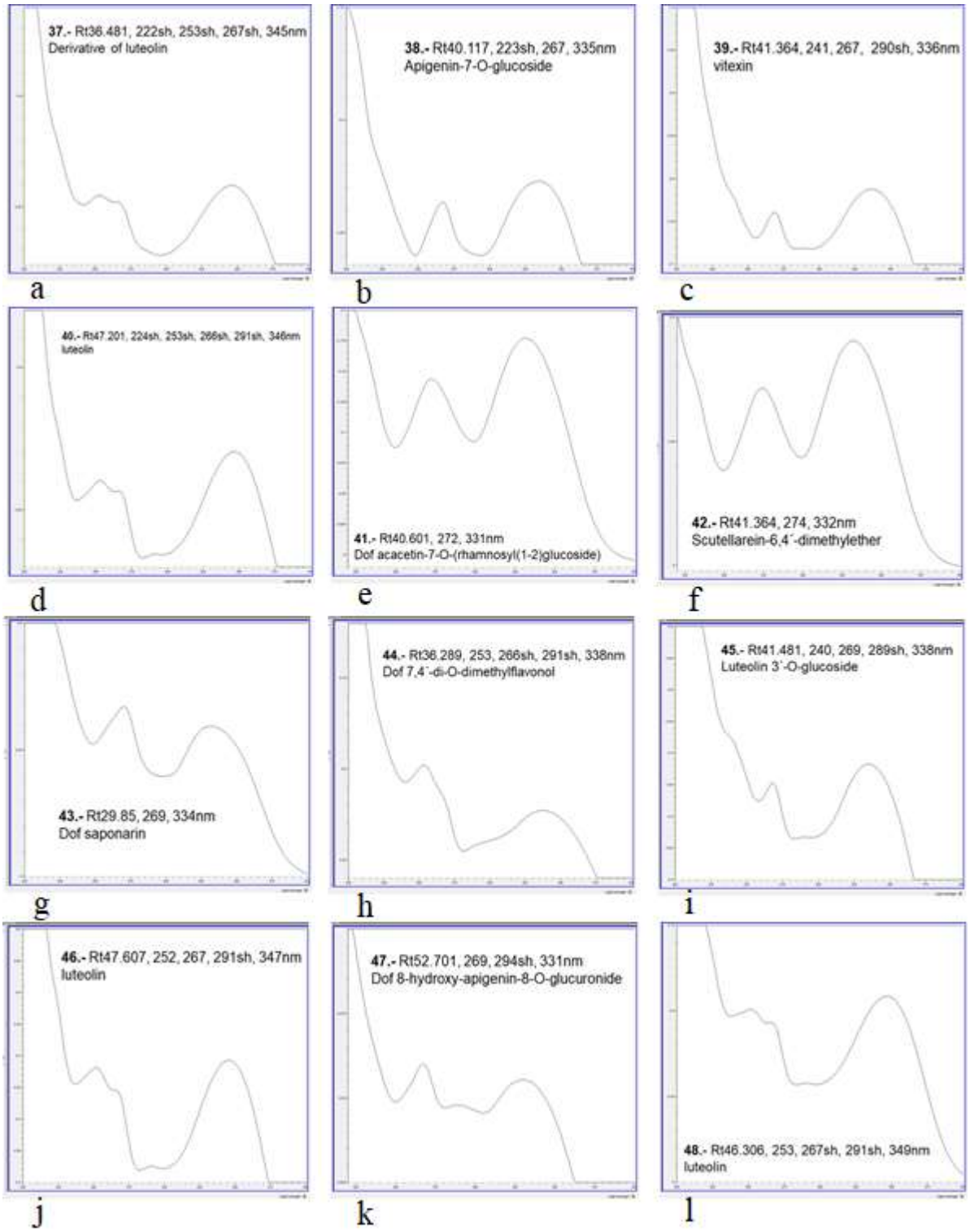

Figure 12a-l. UV spectra, λ_{max}, and retention time of the phenolic compounds 37 to 48 of the foliar tissues

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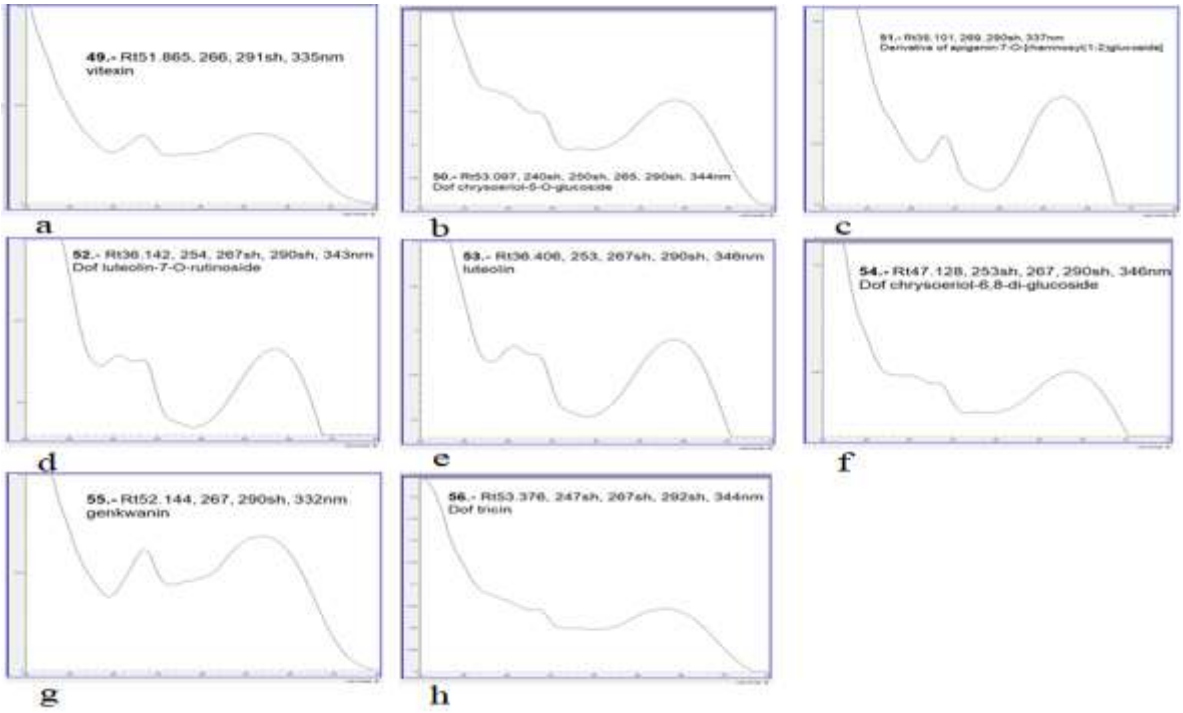

Figure 13a-h. UV spectra, λ_{max}, and retention time of the phenolic compounds 49 to 56 of the foliar tissues

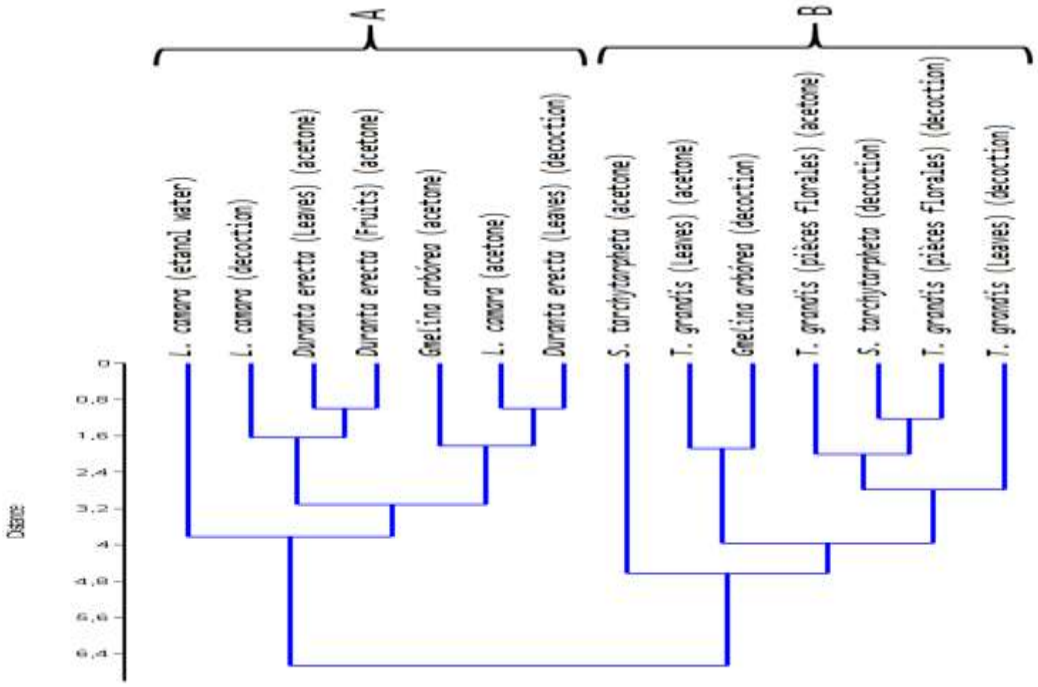

Figure 14. Results of cluster analysis comparing foliar phenol profiles for five varieties Verbenaceae.