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# CHEMICAL EVIDENCE SUPPORTING THE ICLUSION OF AMARANTHACEAE AND CHENOPODIACEAE INTO ONE FAMILY AMARANTHACEAE JUSS. (s.l.)

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## ABSTRACT

*In this study, separation of chemical compounds using Thin layer chromatography technique revealed close relationship between the studied members of the newly constructed family Amaranthaceae Juss. (s.l.). 68% of the calculated affinities between the studied species are above 50% which is an indication for close relationships. 90% is the chemical affinities reported between Chenopodium murale and three species of the genus Amaranthus despite of their great morphological diversity. Among the selected members of the chenopodiaceae, Chenopodium murale and Suaeda monoica are the most closely related species to all of the studied Amaranthaceae . 60%-88% and 54%-88% chemical affinities were reported for the two species with the Amaranthaceae members respectively. GC-Mass analysis of methanolic extracts of the studied species identified 20 compounds common between different species. 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1 and 7-Hexadecenal,(Z)- are the major components common between Amaranthus graecizans, Digera muricata Aerva javanica Gomphrena celosioides of the historical family Amaranthaceae and Suaeda monoica Salsola vermiculata Chenopodium murale Cornulaca monocantha of the historical family Chenopodiaceae, Most of the identified compounds are of pharmaceutical importance such as antioxidants, anti-inflammatory , and Anti-cancerous.*

**KEYWORDS:** *Chemical affinity; TLC; GC- Mass analysis; Amarancaceae; Chenopodiaceae*

## 1. INTRODUCTION

The Chenopodiaceae and Amaranthaceae are morphologically related families of the order Caryophyllales. Plants of these families are characterized by free-central or basal placentation, curved embryos, presence of perisperm, beaked integuments, distinctive phloem plastid morphology, and betalain pigmentation (Judd *et al.*, 2002). Amaranthaceae, and Chenopodiaceae were historically grouped by Bentham and Hooker (1880) in one subclass Monochlamydeaes based on their panporate pollen grains while Engler and Prantl (1887;1898) grouped these families in Archichlamydeae based on some similar petals characters. Hutchinson (1926;

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1959) placed Amaranthaceae, and Chenopodiaceae in the herbaceous group ,the 'Herbaceae'; under the Order Chenopodiales. Takhtajan (1969) kept Amaranthaceae and Chenopodiaceae under Class Magnoliatae, Subclass Caryophyllidae and Super order Caryophyllanae in Order Caryophyllales.

Metcalfe and Chalk (1950) while reviewing the anatomical characters of these families and their taxonomic and phylogenetic positions reported that the Amaranthaceae and the Chenopodiaceae are alike in exhibiting similar anomalous secondary thickening. Similarities between these two families has been confirmed by molecular analysis (Manhart and Rettig 1994; Downie and Palmer 1994; Downie *et al.* 1997; Cuénoud *et al.* 2002). Recent molecular phylogenetic research strongly suggests the inclusion of Chenopodiaceae in Amaranthaceae to form the extended family Amaranceaceae which contains 10 subfamilies, 180 genera, and approximately 2,500 species (APGII, 2003, APGIII, 2009) .

In Sudan, Amaranthaceae is represented by 16 genera which are separately treated by Andrews (1952) as genera of the historical Amaranthaceae and Chenopodiaceae. Many species were reported as weeds of central Sudan (Braun *et al.*, 1991) and common species in northern Sudan (Bebawi and Neugebbohrn , 1991). Recently,( Darbyshire *et al.,* 2015) adopted the molecular classification of the extended family Amaranthaceae and reported their updated names in the annotated checklist for plants of Sudan and South Sudan.

The aim of this paper is to evaluate the significance of biochemical affinities between twelve selected species from the historical families Amaranthaceae and Chenopodiaceae which are recently grouped into one family Amaranthaceae Juss. (s.l.).

## 2. MATERIALS AND METHODS

Twelve plant species belonging to eight genera from the family Amaranthaceae Juss. (s.l.) were selected for this study. They were collected from their natural habitats in Sudan. These are *Amaranthus viridis, Amaranthus graecizans, Amaranthus spinosus, Amaranthus blitum, Digera muricata, Aerva javanica, Gomphrena celosioides* from the historical family Amaranthaceae and *Suaeda monoica, Salsola imbricate, Salsola vermiculata, Chenopodium murale,* and *Cornulaca monocantha* from the historical family Chenopodiaceae . Three grams of dried leaves of each plant were extracted with aqueous methanol and kept for 24 hr in order to get concentrated extract. Three drops of the extracts were used for separation of compounds using Thin layer Chromatography (TLC ) technique in which ethyle acetate-formic acid-glacial acetic acid-water (100:11:11:26) solvents system was used. Retention factors ($R_f$) values of the separated compounds were calculated. Biochemical affinities (PA) between the different species were calculated from the TLC plate following the method adopted by Ellison *et al.* (1962) as the ratio of the number of spots common in each pair of species to the total number of spots separated for the same pair. Three concentrated common spots were separately collected from the TLC plate using a spatula. Each spot was extracted with methanol and filtered to remove the silica. Extracts were subjected to Gas Chromatography and Mass Spectroscopy (GC-MS) analysis for the determination of bioactive volatile compounds. GC-MS analysis of the samples was carried out using Shimadzu Make QP-2010 with non-polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at $40^0$C and held for 3 min and the final temperature of the oven was $480^0$C with rate at 100C [min.sup.1]. 2-µL samples were injected with split less mode. Mass spectra were recorded over 35 - 650 amu range with electron impact ionization energy 70 eV. The chemical components were identified by comparing the retention times of chromatographic peaks using Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

## 3. RESULTS

Screening for compounds using TLC technique, resulted in many spots of different Retention Factor (Rf) values (plate1). Percentage of the paired affinity (PA) based on separated compounds are presented in (table1)**.** 68% of the calculated affinities between the studied species are above 50%. The highest PA values (77% -90%) was recorded between *Chenopodium murale* and the Amaranthaceae species. 60%-88% and 54%-88% chemical affinities were reported between *Suaeda monoica* and the Amaranthaceae species respectively.

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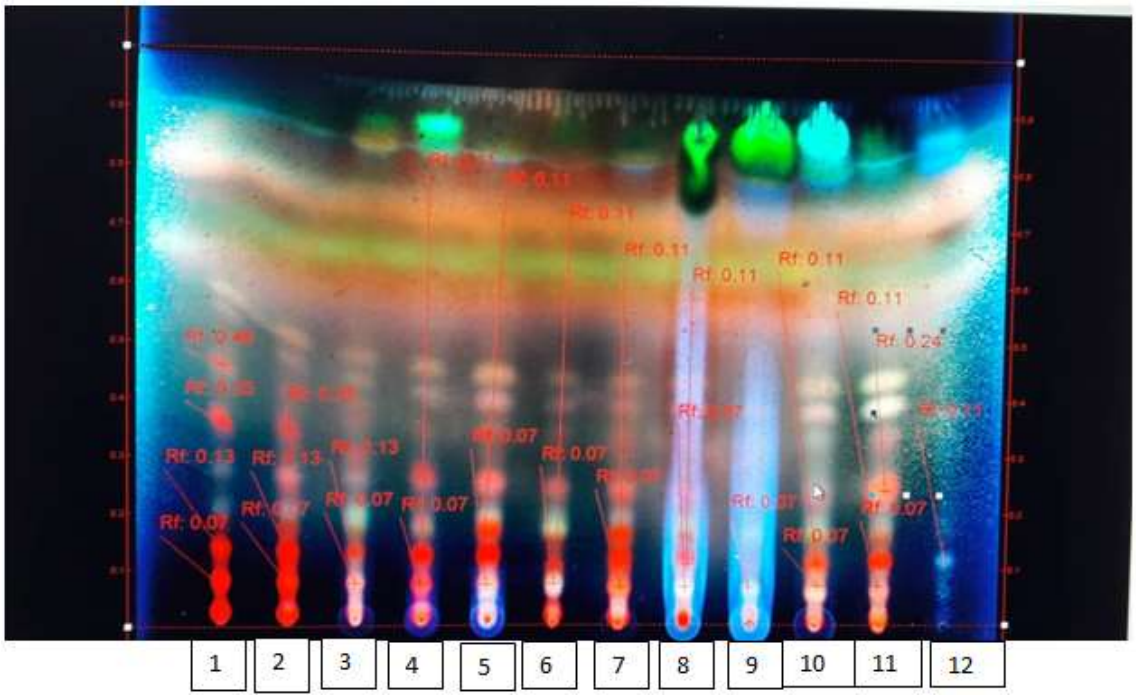


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**Plate 1.** TLC chromatogram of methanolic extract of leaves of the studied species: (1) *Amaranthus viridis* (2) *Amaranthus graecizans* (3)*Amaranthus spinosus* (4)*Amaranthus blitum* (5) *Digera muricata* (6) *Aerva javanica* (7) *Gomphrena celosioides (8) Suaeda monoica (9) Salsola imbricate(10) Salsola vermiculata (11) Chenopodium murale (12) Cornulaca monocantha*

**Table1. Percentage of the paired affinity (PA) based on all of the compounds separated on TLC for the studied species**

| Species | *%age paired affinities* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***1*** | ***2*** | ***3*** | ***4*** | ***5*** | ***6*** | ***7*** | ***8*** | ***9*** | ***10*** | ***11*** | ***12*** |
| ***1*** | 100 | 86 | 55 | 40 | 55 | 40 | 75 | 72 | 44 | 54 | 77 | 42 |
| ***2*** | | 100 | 45 | 36 | 50 | 66 | 54 | 60 | 57 | 63 | 90 | 50 |
| ***3*** | | | 100 | 55 | 66 | 44 | 66 | 77 | 44 | 72 | 80 | 44 |
| ***4*** | | | | 100 | 86 | 86 | 87 | 87 | 57 | 88 | 88 | 50 |
| ***5*** | | | | | 100 | 66 | 77 | 88 | 37 | 80 | 90 | 44 |
| ***6*** | | | | | | 100 | 87 | 87 | 57 | 77 | 88 | 44 |
| ***7*** | | | | | | | 100 | 88 | 62 | 72 | 90 | 44 |
| ***8*** | | | | | | | | 100 | 80 | 50 | 62 | 80 |

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| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***9*** | | | | | | | | | 100 | 37 | 25 | 50 |
| ***10*** | | | | | | | | | | 100 | 97 | 28 |
| ***11*** | | | | | | | | | | | 100 | 42 |
| ***12*** | | | | | | | | | | | | 100 |

***Key to the species****: (1) Amaranthus blitum (2) Amaranthus viridis (3) Digera muricata (4) Aerva javanica (5) Amaranthus graecizans (6) Gomphrena celosioides (7) Amaranthus spinosus (8) Suaeda monoica (9) Salsola imbricate (10) Salsola vermiculata (11) Chenopodium murale (12) Cornulaca monocantha*

Table 2 shows common concentrated spots separated from extracts of different plants. Gc-Mass analysis of the common spots identified 20 organic compounds. GC-Mass chromatograms of the detected compounds were represented in figures 1, 2, and 3. *Aerva javanica, Amaranthus graecizans, Gomphrena celosioides , Digera muricata , Suaeda monoica, Salsola vermiculata., Chenopodium album,* and *Cornulaca monocantha* have a common spot of Rf value 0.011. GC-mass analysis of this spot identified nine compounds of which 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1 and 7-Hexadecenal,(Z)- are the major components. 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) represents the major component identified for the spot of the Rf value 0.13 which is separated for *Amaranthus blitum. , Amaranthus viridis,* and *Amaranthus spinosus*. The spot of Rf value 0.35 is reported for both *Amaranthus blitum* and *Amaranthus viridis* 4-Hydroxy-2-methylbenzaldehyde represents the majoir component of this spot which contains 8 other compounds.

**Table1. Concentrated spots selected for GC-Mass analysis**

| ***Plant species*** | **$R_f$ values of the separated compounds** | | |
|---|---|---|---|
| | 0.11 | 0.13 | 0.35 |
| *Amaranthus blitum* | - | + | + |
| *Amaranthus viridis* | - | + | + |
| *Amaranthus spinosus* | - | + | - |
| *Amaranthus graecizans* | + | - | - |
| *Digera muricata* | + | - | - |
| *Aerva javanica* | + | - | - |
| *Gomphrena celosioides* | + | - | - |
| *Suaeda monoica* | + | - | - |
| *Salsola imbricata* | - | - | - |
| *Salsola vermiculata* | + | - | - |
| *Chenopodium murale* | + | - | - |
| *Cornulaca monocantha* | + | - | - |

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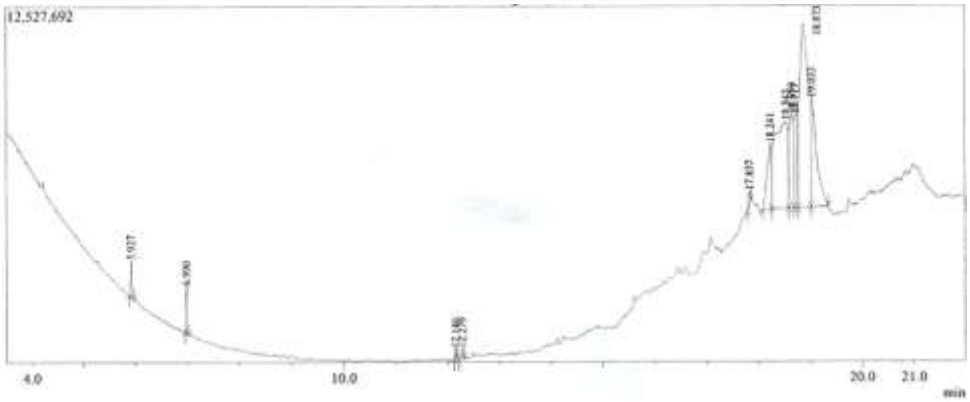


**Figure 1. GC-MS chromatogram of Phytoconstituents obtained from spot Rf value 0.11**

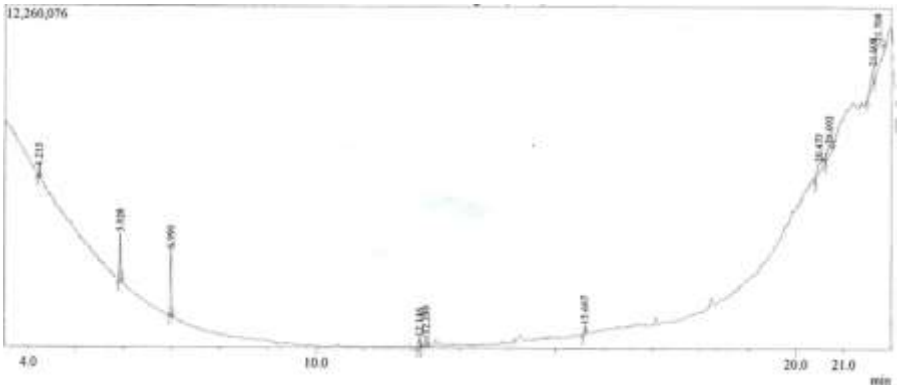


**Figure 2. GC-MS chromatogram of Phytoconstituents obtained from spot Rf value 0.13**

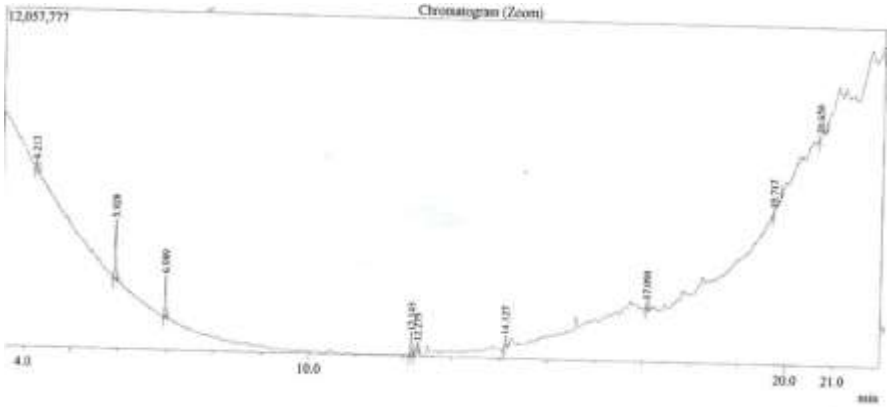


**Figure 3. GC-MS chromatogram of Phytoconstituents obtained from spot Rf value 0.35**

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**Table3. Phytochemical constituents identified for the studied species**

| Rf values | Identified compounds | R.Time | Area% |
|---|---|---|---|
| 0.11 | Benzaldehyde,4-methoxy- | 5.927 | 0.93 |
| | 3,4-Dimethoxyphenylacetone | 6.990 | 1.14 |
| | Hexadecanoic acid,methyl ester | 12.146 | 0.31 |
| | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien | 12.279 | 0.36 |
| | 7-Hexadecenoic acid,methyl ester,(Z)- | 17.837 | 0.55 |
| | Di-n-octyl phthalate | 18.241 | 6.31 |
| | 7-Hexadecenal,(Z)- | 18.542 | 25.31 |
| | Octadecanoic acid,1-{{(1-oxohexadecyl)oxy}r | 18.717 | 7.06 |
| | 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1 | 18.873 | 35.71 |
| 0.13 | Benzaldehyde, 4-methoxy- | 5.928 | 10.50 |
| | Benzeneacetic acid, 3,4-dimethoxy-, methyl es | 6.990 | 12.88 |
| | Hexadecanoic acid, methyl ester | 12.146 | 2.56 |
| | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien | 12.280 | 3.35 |
| | Azacyclotridecan-2-one | 15.607 | 2.65 |
| | Patchouli alcohol | 20.692 | 13.80 |
| | 6-Octen-1-ol,3,7-dimethyl-,propanoate | 21.608 | 11.05 |
| | 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) | 21.708 | 32.02 |
| 0.35 | Undecane | 4.213 | 4.10 |
| | 4-Hydroxy-2-methylbenzaldehyde | 5.928 | 29.45 |
| | Benzeneacetic acid , 3,4-dimethoxy-,methyl es | 6.989 | 19.15 |
| | Hexadecanoic acid, methyl ester | 12.145 | 13.10 |
| | Butylated Hydroxytoluene | 12.279 | 6.07 |
| | Methyl stearate | 14.127 | 7.67 |
| | Phenol,2,2-methylenebis{6-(1,1-dimethylethy | 17.090 | 4.99 |
| | Linoleic acid ethyl ester | 19.717 | 5.66 |

## 4. DISCUSSION

In this study, identification of nine phytochemical compounds common between *Amaranthus graecizans, Digera muricata Aerva javanica Gomphrena celosioides* of the historical family Amaranthaceae and *Suaeda monoica Salsola vermiculata Chenopodium murale Cornulaca monocantha* of the historical family Chenopodiaceae, supports the new inclusion of the two families by the APG III ( APG, ) into a new broadened family Amaranthaceae Juss. (s.l.). According to Ellison *et al.* (1962) PA values of 50% and above are considered as marker of close relationship. The results revealed 90% chemical affinities between *Chenopodium murale* and three species of the genus *Amaranthus* despite of their great morphological diversity. Among the selected members of the *chenopodiaceae, Chenopodium murale* and *Suaeda monoica* are the most closely related species to all of the studied Amaranthaceae . 60%-88% and 54%-88% chemical affinities were reported for the two species with the Amaranthaceae members respectively. Most of the identified compounds are of pharmaceutical importance. Phenol,2,2-methylenebis{6-(1,1-dimethylethy), 7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-dien, Butylated Hydroxytoluene, and 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) reported by many authors as antioxidants (Hema et al, 2011; Sudharsan *et al*, 2010; Naher *et al*, 2013). Hexadecanoic acid, methyl ester, Linoleic acid ethyl ester , Hexadecanoic acid, methyl ester, 3-Cyclohexen-1-ol, and 1-methyl-4-(1-methylethyl) reported as anti-inflammatory (Hema et al, 2011; Naher et al, 2013; Othman *et al*, 2015; Sudha *et al*, 2013 ). Benzaldehyde, 4-methoxy, Methyl stearate, Butylated Hydroxytoluene, 7-Hexadecenal,(Z)-, and 3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) considered as Anti-cancerous as reported by Kundu and Metra (2016), Naher *et al* (2013), Ukwubile *et al* (2019), Hamid *et al*, (2017). Antibacterial activity of the Patchouli alcohol3-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl) 7-Hexadecenoic acid,methyl ester,(Z)- Di-n-octyl

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phthalate Octadecanoic acid,1-{{(1-oxohexadecyl)oxy}r, and Di-n-octyl phthalate has been reported by Bunrathep et al, (2006), Naher et al, (2013), Krishnaveni et al., (2014). Kale ,(2015) and Jabeen , (2018).

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