

Phenolics compounds from Amaranthaceae family: extraction and biological properties

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Abstract

Species of the Amaranthaceae family have become a potential group of plants for their latent beneficial properties. Their use in traditional medicine and potential biological properties can be considered as the basis to guide further investigations about their characteristics and beneficial uses. In this work, three species of the Amaranthaceae family traditional from China (*Alternanthera sessilis* L., *Dicliptera chinensis* L. and *Dysphania ambrosioides* L.) were proposed as an alternative source of bioactive compounds, namely phenolic compounds (Adegbola et al., 2020).

The study was aimed to extract and characterize the phenolic compounds of the three species and search for possible cytotoxic and antimicrobial activities. For this purpose, the antimicrobial activity was tested against Gram (-), Gram (+) pathogenic bacteria. The antitumor properties were assessed on *in vitro* studies to assess the inhibition of the growth of several tumor cell lines. Thus, Amaranthaceae family could be an alternative source of bioactive compounds to formulate new innovative products and incorporate them into the food, cosmetic and pharmaceutical industry.



Alternanthera sessilis *Dicliptera chinensis* *Dysphania ambrosioides*

Introduction

The search of new bioactive compounds to produce drugs is a necessity promoted by the increase of resistant bacteria, new viruses and numerous pathologies without treatment. The production of drugs produced, in 2016, 250 billion euros, data that situates the pharmaceutical industry in one of the most rentable markets. One of the most used sources of bioactive compounds are the natural products. Since 1981 was approved 1881 new drugs of which the 25% came from natural sources and the 41.9% came from no synthetic sources (McKerrow, 2015) (Figure 1).

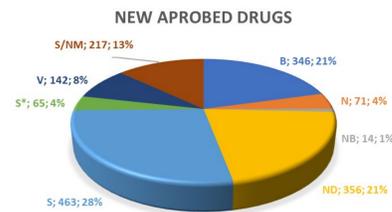
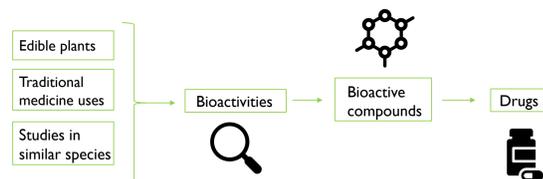


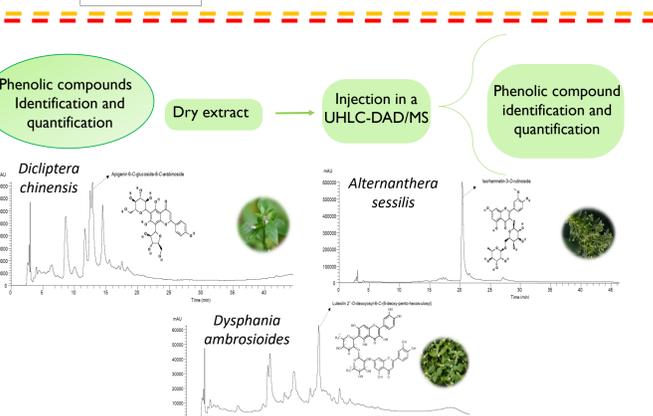
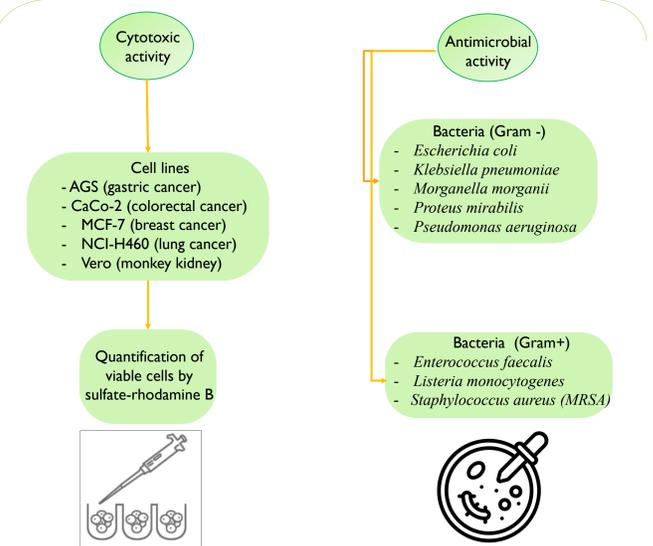
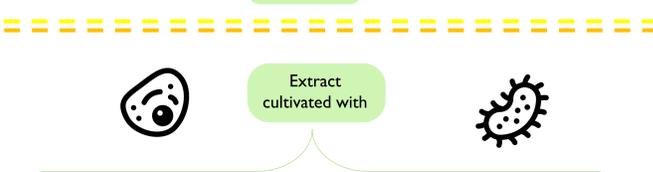
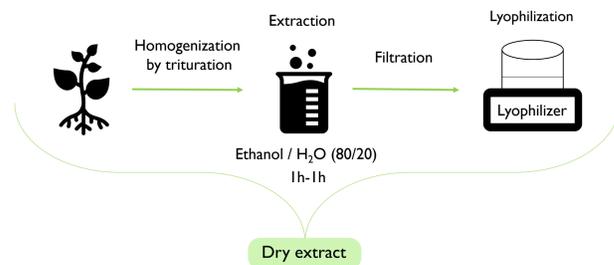
Figure 1. Drugs approved since 1981 (n = 1881) classified by their source. B: biological macromolecule, N: unaltered natural product, NB: botanical drug, ND: natural product derivative, S: synthetic drug, S*: Synthetic drug (NP pharmacophore), V: vaccine, S/NM: mimic of natural products.

One of the biggest sources of natural drugs is the secondary metabolites of the plants. Inside this big group, phenolic compounds are the most important for their bioactivities, diversity and abundance. The three species under study have been present in the diets of Asian and middle East countries. Moreover, these plants have been used in traditional medicine for treat different pathologies an illness (skin diseases, ocular diseases, wound healing, animal bites etc.). This use could be a clue of the presence of bioactive compounds. Therefore, this work consisted in the identification of the phenolic compounds present in the species and their bioactivities.



Methodology

This work can be separated in three sections. First of all, was made the extraction of the phenolic compounds by maceration. The result of this step is a dry extract ready to continue with the biological activities.



Results

The data obtained showed interesting results in the cytotoxic (Table 1) and antimicrobial activity (Table 2). Moreover, in Table 3 is represented all the compounds identified and their concentration in the samples.

Table 1. The cytotoxic (GI₅₀ µg/mL) activities.

Cell Lines	<i>D. chinensis</i>	<i>A. sessilis</i>	<i>D. ambrosioides</i>
Caco-2	>400	>400	188±14
MCF-7	>400	>400	245±13
NCI-H460	>400	>400	263±12
AGS	>400	>400	263±22
Vero	>400	>400	>400

Table 2. The antimicrobial activity expressed in MIC and MBC (mg/mL) of the different microorganism tested.

Antimicrobial activity	<i>Dicliptera chinensis</i>		<i>Alternanthera sessilis</i>		<i>Dysphania ambrosioides</i>	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria						
<i>Escherichia coli</i>	20	>20	10	>20	10	>20
<i>Klebsiella pneumoniae</i>	>20	>20	20	>20	>20	>20
<i>Morganella morganii</i>	10	>20	5	>20	5	>20
<i>Proteus mirabilis</i>	>20	>20	>20	>20	>20	>20
<i>Pseudomonas aeruginosa</i>	>20	>20	>20	>20	>20	>20
Gram-positive bacteria						
<i>Enterococcus faecalis</i>	10	>20	20	>20	10	>20
<i>Listeria monocytogenes</i>	10	>20	>20	>20	10	>20
MRSA	10	>20	5	>20	10	>20

Table 3 Tentative identification of the phenolic profile and their quantification (mg/g of dry extract).

RT	Tentative identification	Samples		
		<i>D. chinensis</i>	<i>A. sessilis</i>	<i>D. ambrosioides</i>
4.88	3-p-Coumaroylquinic acid	1.2±0.1	nd	nd
6.40	p-Coumaroyl pentoside acid	nd	0.33±0.01	nd
6.66	Caffeic acid acetylhexoside	nd	0.75±0.04	nd
6.87	Sulfo-caffeic acid	1.17±0.05	nd	nd
8.59	Luteolin-6-C-glucoside-7-O-glucoside	nd	0.31±0.02	nd
8.61	Apigenin-6,8-di-C-glucoside (vicenin-2)	2.0±0.1	nd	nd
10.10	Luteolin-6-C-hexosyl-8-C-pentosyl	0.606±0.004	nd	nd
11.30	Quercetin-3-O-glucosyl-pentoside-7-O-glucuronide	nd	0.535±0.01	nd
11.70	Apigenin-6-C-xyloside-8-C-glucoside	1.3±0.1	nd	nd
12.50	Apigenin 2"-O-xyloside-8-C-hexoside	1.9±0.1	nd	nd
12.80	Dihydroxyl methyl quercetin-chalcone	nd	0.56±0.01	nd
12.81	Apigenin 6-C-glucoside-8-C-arabinsoside (Schafoside)	2.40±0.04	nd	nd
13.60	Luteolin 2"-O-deoxyhexosyl-6-C-glucoside	nd	0.59±0.04	nd
13.84	5-Hydroxy-3,4' 7 trimethoxy-flavone	nd	nd	0.93±0.01
13.92	Luteolin-6-C-glucoside	nd	1.6±0.1	nd
14.53	Apigenin-6-C-glucoside-8-C-arabinsoside	2.0±0.1	nd	nd
15.20	Eriodictyol-O-glucuronide	nd	nd	0.001±0.00002
15.45	Isorhamnetin-3-O-neohesperidoside	nd	nd	1.13±0.01
16.30	Luteolin 2"-O-deoxyhexosyl-C-pentoside	nd	0.247±0.004	nd
16.31	Kaempferol dirhamnoside-O-hexoside	nd	nd	0.98±0.05
16.57	Quercetin-3-O-rutinoside	nd	0.56±0.01	0.721±0.002
16.94	Lignan-O-coumaroylglucoside	nd	nd	0.337±0.005
17.01	Apigenin 6-C-pentosyl-8-C-hexoside	0.35±0.01	nd	nd
17.26	Quercetin-O-rhamnosyl-pentoside	nd	nd	0.888±0.001
17.30	Apigenin-6-C-glucoside	nd	0.168±0.02	nd
17.50	Apigenin-6-C-hexoside-8-C-rhamnoside	0.32±0.02	nd	nd
17.52	Chrysoeriol-8-C-(2-rhamnosyl)hexoside	nd	0.01±0.002	nd
17.59	Kaempferol-O-rhamnosyl-O-pentoside	nd	nd	0.848±0.01
17.90	Kaempferol-O-rhamnoside-O-hexoside	nd	0.77±0.02	nd
18.00	Kaempferol-O-rhamnoside-O-hexoside	nd	0.93±0.01	nd
18.12	Isorhamnetin-3-O-neohesperidoside	nd	nd	0.68±0.003
18.40	Apigenin-8-C-rhamnoside-6-C-glucoside	nd	0.23±0.02	nd
18.42	Apigenin-6-C-arabinsoside-8-C-glucoside	0.28±0.02	nd	nd
19.00	Luteolin-7-O-neohesperoside	nd	0.73±0.05	nd
19.75	Luteolin-7-O-Rhamnosyl(1-2)hexoside	nd	nd	0.67±0.01
19.90	Apigenin-6-C-glucoside-8-C-xyloside	0.08±0.01	nd	nd
20.00	Luteolin-O-rutinoside	nd	0.747±0.02	nd
20.37	Isorhamnetin-3-O-rutinoside	nd	nd	5.75±0.04
20.60	Luteolin 2"-O-deoxyhexosyl-6-C-(6-deoxy-pentohexoside-ulosyl)	nd	0.705±0.04	nd
20.61	Methyl-luteolin 2"-O-deoxyhexosyl-6-C-hexoside	nd	0.8±0.1	nd
21.53	Kaempferol-O-rhamnosyl-O-pentoside	nd	nd	0.587±0.002
22.10	Luteolin-8-C-(rhamnosyl)ketodeoxihexoside	nd	2.02±0.04	nd
22.80	Luteolin-8-C-(rhamnosyl)ketodeoxihexoside	nd	0.29±0.01	nd
23.20	Luteolin-O-deoxyhexosyl-C-deoxy-pento-hexosulosyl	nd	1.0±0.1	nd
23.80	Luteolin-O-deoxyhexosyl-C-deoxy-pento-hexosulosyl	nd	0.91±0.003	nd
24.60	Apigenin-6-C-glucoside-2"-O-rhamnoside	nd	0.42±0.01	nd
24.67	Kaempferol-O-hexose-O-galic acid	nd	nd	0.707±0.003
25.50	Apigenin-4"-O-hexoside-D-deoxyhexoside	nd	0.90±0.01	nd
27.11	Acetylated luteolin pentosyl-rhamnoside	nd	nd	0.81±0.02
Total Phenolic compounds		13.7±0.5	16±1	15.0±0.1

Conclusion

The results showed significant results in antimicrobial and cytotoxic activity.

The antimicrobial activity is not strong enough to think in a father investigation in terms of search a possible antibiotic in this species. Nevertheless, the MIC of *Dicliptera chinensis* and *Alternanthera sessilis* are interesting to use these extracts as a possible natural food preservative that could increase the shelf life of the food thanks to the inhibition of microbial growth.

Dysphania ambrosioides present a low IC₅₀ against CaCo, MCF-7 and NCI-H460 cell lines. This activity could be useful for find new bioactive compounds for new treatments against different cancers.

Further investigations are needed to complete this study and found what are the compounds in this species that give to this species their biologic activities.



Protection



New anticancer treatments

References

Adegbola, P. I., Adetutu, A., & Olaniyi, T. D. (2020). Antioxidant activity of *Amaranthus* species from the Amaranthaceae family – A review. *South African J. Bot.*, 133, 111–117.

McKerrow, J. H. (2015). Recognition of the role of Natural Products as drugs to treat neglected tropical diseases by the 2015 Nobel prize in physiology or medicine. *Nat. Prod. Rep.*, 32, 1610–1611.

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