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Orientadora

Dedication

I dedicate this thesis to my loving family, whose unwavering support, encouragement and sacrifices have been the bedrock of my academic journey.

I am also grateful to my dedicated supervisor, Prof. Déborah Yara Alves Cursino dos Santos, whose knowledge, guidance, confidence and inspiration have shaped my intellectual growth. I owe her a debt of gratitude.

Read in the name of your GOD (ALLAH) who created Human beings from a clinging substance. Read and your Lord is the most Generous - Who taught by the pen - Taught man that which he knew not."

Quran
(Surah Al-'Alaq, 96:1-5)

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GENERAL INTRODUCTION

Introduction

Biological and chemical interactions among organisms are the essential determinants of the communities' habitat (Gebrekiros, 2016). This interaction may be for basic requirements like space, light or energy, water and nutrients (nitrogen, carbon, potassium and phosphorus etc.). (Blum & Blum, 2011).

An Austrian Professor called Hans Molisch was the first biologist mentioned the allelopathy term in his book "Der Einfluss einer Pflane uaf die andere-alleopathie" (Molisch, 1937). He defined it as "an inhibitory chemical compounds which are released from one plant that affects other plant". However, in 1969 the term 'allelopathy' was defined by the International Allelopathy Society (IAS) as "any biological interaction involving secondary metabolites produced by plants, algae, bacteria, fungi and viruses (exceptional case) that influences directly or indirectly the growth and development of nearly associated plants" (Jabran & Jabran, 2017). These influences can involve photosynthesis, respiration, growth and development, transpiration rates, cell division, enzymes activity, nutrients uptake, impaired phytohormone metabolism, gene expression, changes to membrane permeability and the production of reactive oxygen species (ROS) through the release of allelochemicals (Zhu & Yu, 206).

Allelopathy involves the release of plant secomdary metabolites formally known as allelochemicals produced by plants, fungi, bacteria and algae that influences the germination, growth and development of other neighboring plants/crops (Weir *et al*., 2004). These allelochemicals also work on communication and defense against herbivores and competitors (Bacellar & Vermelho, 2013). These chemicals can have profound effects on the growth and development of neighboring plants (in case of plant-plant interaction), either inhibiting or promoting their growth and development, depending on the concentration and type of allelochemicals. For example, allelopathic compounds such as phenolics, terpenoids, and alkaloids affect mostly negatively seed germination, root growth and nutrient uptake of other plants, ultimately influencing plant community structure and diversity (John & Sarada, (2012).

In allelopathic interactions the donor plant releases such allelochemicals that need to persist in the soil and reach sufficient concentration to cause its phytotoxic effect over the target species called receiver plants (Duke, 2015). Chemicals with allelopathic potential are present in nearly all plants in their respective tissues, including leaves, stems, roots, flowers, seeds, bark, and buds. Under the appropriate environmental conditions, these phytotoxins may be released into the environment in sufficient quantities to affect the growth and development of

neighboring plants (Liebman & Davis, 2000). Understanding the sources, mechanisms of action, and ecological implications of allelochemicals are crucial for developing effective strategies for sustainable agriculture and ecosystem management (Meiners *et al*., 2012).

Recently, allelopathy is of key importance in agriculture by using allelopathic plants as tools to suppress noxious weeds, which improve soil quality and crops yield as cover crops, mulch, green manures and/or grown in rotation (Khalil *et al*., 2010). However, this ability to suppress or/and control weeds depends on the concentration of the chemical compounds released into the soil. Allelochemicals at high concentrations may be more effective; however, at lower concentrations these allelochemicals may sometimes stimulate the growth of other species (Bhowmik, 2003).

According to Rice (1984) and Putnam (1985), there are different ways of releasing allelochemicals (Figure 1): (1) Leaching is the process of allelochemical brought by means of water. For example, rainfall, dew or irrigation may leach the phytotoxic chemicals from the aerial parts of the plants and subsequently deposited on the other plants or in the soil, which affect the growth and development of nearby plants. The phenomenon of leaching may also occur through plants residue (Hussain *et al*., 2010); (2) The allelochemical can be released into the atmosphere, mostly for arid and semi-arid conditions. The compounds may be absorbed in the vapors form to the nearby plants, absorbed from condensed in dew or may reach the soil and taken up by roots (Dirk *et al*., 2016); (3) The toxic substances can be released upon decomposition of plant material and/or as products generated by micro-organism utilizing the residue (Kensa, 2011); (4) Root exudates are an important pathway for the release of allelochemicals, as these compounds can directly interact with the roots of neighboring plants (Weston, 2003).

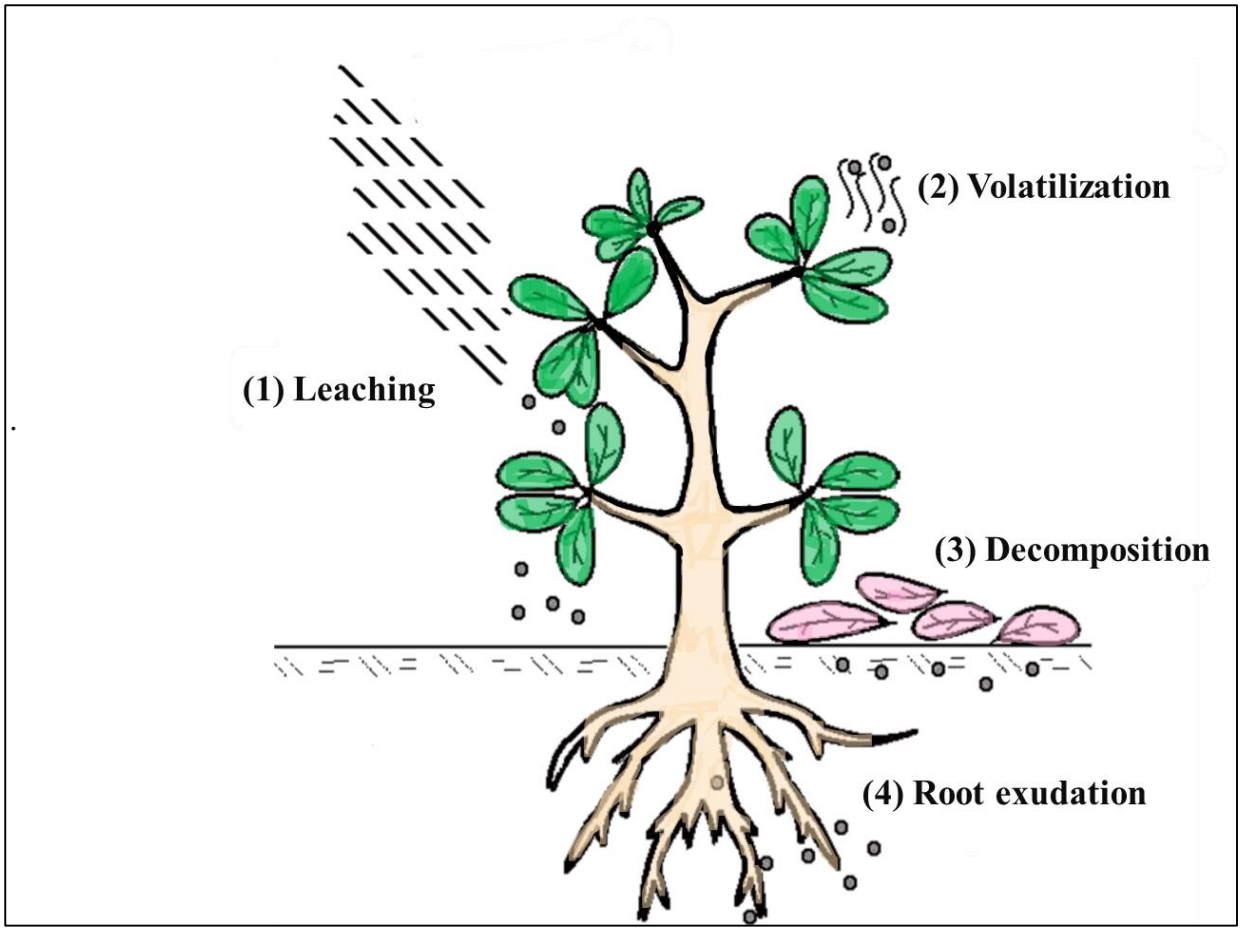


Figure 1. Different processes are involved in the release of allelochemicals into the environment. These are (1) leaching, (2) volatilization, (3) decomposition and (4) root exudation through which different allelochemicals released to environment (Albuquerque *et al*., 2011).

Kopsell & Kopsell (2006) stated that several factors affect allelochemical production in plants including pH, temperature, light, water stress, mineral deficiency, and age of plant. Mølmann *et al.* (2015), for example, claim that there is a link between contents of glucosinolates and long photoperiod, whereas concentrations of gluciberin and glucoraphanin significantly increased in *Brassica oleracea* L. tissues under long day photoperiod. Glucosinolate also increases in *Brassica rapa* L. after increasing the temperature (Justen & Fritz, 2013). According to Taiz and Zeiger (2010), water stress affects physiology, morphology and chemistry of plants, which can in turn affect root and shoot growth and production of allelochemicals. Karageorgou *et al*. (2002) found that the concentration of phenolic compounds in leaf exudate of *Dittrichia viscosa* L. was increased under drought stress, even though the total phenolics were reduced because of reduced leaf area.

Once reaching the target plants, allelochemicals can lead to changes in various growth parameters, including seeds germination, root and shoot length, biomass accumulation and nutrient uptakes (Cheng & Cheng, 2015). On the same way, different allelochemicals released by donor plants can affect physiological processes such as, seed germination, cell division and hormone regulation in recipient plants (Meiners *et al*., 2012).

Muhammad & Majeed (2014) studied the allelopathic potential of fresh aqueous extract (FAE) and air-dried aqueous extracts (DAE) from the root, shoot and leaves of sunflower (*Helianthus annuus* L.) on the germination and seedling growth of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). Results showed seed germination, growth, and dry biomass of seedlings of wheat and maize were significantly reduced by water extracts from all plant parts. The leaf aqueous extract significantly decreased seed germination of wheat by 15.21%, plumule and radical growth were reduced by 21.66 and 28.44%, suggesting that leaves are the major reservoir of allelochemicals among the other parts. Similarly, Turk *et al*. (2005) evaluated the allelopathic effects of different plant parts (leaf, stem, flower, and root) of black mustard (*Brassica nigra* L.) on radish (*Raphanus sativus* L.) germination and seedling growth. They found that radish seed germination and seedling growth were inhibited significantly by all aqueous extracts, with leaf extract causing maximum inhibition, followed by root and stem extracts.

Studies have demonstrated the allelopathic effect on target plant's physiology, including photosynthesis, antioxidant enzymes, seed germination and growth traits (Farhoudi & Lee, 2013; Cheng & Cheng, 2015). Upon exposure to allelochemicals, the target plants exhibit a rapid production of reactive oxygen species (ROS) in the form of superoxide radicals ($O2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) (Bais *et al*., 2003). To trigger a cascade of physiological responses to scavenge these ROS and ultimate damaging effects, the activity of key antioxidant enzymes, such as superoxide dismutase (SOD) (Gupta *et al*., 2018), peroxidase (POD) (Zeng *et al*., 2001) and ascorbic acid peroxidase (APX) (Zuo *et al.*, 2022), undergoes alteration to mitigate the oxidative stress. These enzymes play critical roles in the antioxidant defense system of plants. Among all, SOD acts as the first line of defense by converting superoxide radicals into hydrogen peroxide (H_2O_2), while POD and APX are involved in the detoxification of H_2O_2 and other peroxides (Ighodaro *et al*., 2018). The regulation of antioxidant enzyme activity is a vital adaptive response to oxidative stress, allowing plants to maintain cellular homeostasis and protect themselves from oxidative damage. The modulation of these enzymes in response to oxidative stress represents an

essential strategy employed by plants to counteract the harmful effects of ROS and ensure their survival under stressful conditions (Ghori *et al*., 2019).

Batish *et al*. (2008) reported the activities of proteases, POD, and polyphenol oxidases (PPOs) during root development in mung bean (*Phaseolus aureus* Roxb.) hypocotyl cuttings exposed to caffeic acid. A decrease in the content of total endogenous phenolics was observed, suggesting a complex modulation of enzymatic and biochemical processes in response to allelochemical exposure.

The impact of allelochemicals on the photosynthesis of target plants is characterized by the inhibition or complete damage to the photosynthetic machinery, leading to the decomposition of photosynthetic pigments (Cheng & Cheng, 2015). Consequently, the main photosynthetic pigments such as chlorophyll *a*, chlorophyll *b* and carotenoids are affected, resulting in a decrease in electron transfer, reduced ATP synthesis, and impaired stomatal conductance and transpiration. These combined effects lead to the inhibition of the photosynthesis process (Li *et al*., 2012; Sverdlov *et al*., 2015).

Al-Haithloul *et al*. (2022) monitored the phytotoxic effect of leachates of *Acacia saligna* (Labill.) Wendl. on chlorophyll content in several target plant species, including wheat, barley, arugula, and radish. The treatment with *A. saligna* leachate resulted in a significant decrease in photosynthesis and PSII activities in all target species, affecting their yield production.

Similarly, Al-Wakeel *et al*. (2007) explored the allelopathic effect of *Acacia nilotica* L. leaf residue on shoot length and photosynthetic pigments of *Pisum sativum* L. Their findings revealed that *A. nilotica* leaf residue had a negative impact on photosynthetic parameters Qualitative and quantitative HPLC analysis of water extracts from *A. nilotica* leaves indicated the presence of protocatechuic and caffeic acids as the principal phenolic compounds, accompanied by significant amounts of ferulic acid, cinnamic acid and apigenin. Trace amounts of pyrogallic acid, *p*-cournaric acid, syringic acid, and coumarin were also detected. These findings provided insights into the chemical composition of *A. nilotica* leaves and suggested the potential allelochemicals responsible for their allelopathic effects.

The study of allelopathy holds potential applications in agriculture, forestry and weed management (Macias *et al*., 2007). Understanding the allelopathic potential of crops and weeds can aid in developing sustainable farming practices, such as utilizing cover crops with allelopathic properties to suppress weeds growth and development *via*, environment friendly manner (Otusanya *et al.*, 2012). On the same way, allelopathy also poses as a strong bio-herbicides reducing the reliance on synthetic herbicides (Hassan *et al*., 2023). Furthermore,

allelopathy has implications for agroforestry systems, where certain tree species can release allelochemicals that enhance or inhibit the growth of associated plants, affecting biodiversity and yield (Rizvi *et al*., 1999). Additionally, allelopathic compounds derived from plants have the potential for developing natural biopesticides, which can contribute to environmentally friendly pest management strategies (Kumar *et al*., 2021). Moreover, Huang *et al*. (2003) reported that allelochemicals are effective against pathogens causing plant diseases and infections and have good antifungal activity.

Notwithstanding, allelopathy can also present detrimental effects on plant diversity, crop productivity, nutrient cycling, and ecosystem dynamics (Wardle *et al*., 2011). Allelochemicals released by certain plants can hinder the germination of seeds and impede the growth of neighboring plants, resulting in reduced plant diversity and the suppression of desirable plant species (Meksawat & Pornopromm, 2010). In agricultural sectors, allelopathic interactions can negatively impact crop productivity leading to decreased yields and economic losses for farmers (Li *et al*., 2019). Furthermore, allelopathy can disrupt nutrient cycling in soil by interfering with the availability and uptake of essential nutrients by plants. This can cause nutrient deficiencies and imbalances within plant communities (Kumar *et al*., 2013). Additionally, certain invasive plant species present an allelopathic mechanism that reinforce their invasiveness. The allelochemicals produced give them a competitive advantage over native plants, leading to the displacement of native species and the alteration of ecosystems. These negative impacts highlight the importance of understanding and managing allelopathic interactions to preserve plant diversity, ensure sustainable crop production, and maintain the ecological balance of ecosystems (Lundkvist & Verwijst, 2011).

Separation of *Senegalia* and *Acacia*

Previous phylogenetic studies have described *Acacia* as a polyphyletic genus encompassed a broad and varied group of plants, including trees and shrubs, primarily distributed in Africa but also occurring in other regions worldwide (Kyalangalilwa *et al*., 2013). Additionally, molecular data also corroborates that *Acacia* is polyphyletic, indicating that this genus did not form a single evolutionary lineage but consisted of multiple lineages that were distantly related to one another.

According to research carried out by Kyalangalilwa *et al*. (2013), genetic markers such as chloroplast DNA *trn*L-*trn*F region, ribosomal DNA, and satellite markers, have been utilized to investigate the genetic divergence within the *Acacia* genus and support the formation of new genera. For instance, the interpretation of chloroplast DNA *trn*L-*trn*F region sequences

provided an interesting example of genetic divergence. Researchers examined samples of *Acacia* from non-Australian regions (non-native range) and identified significant genetic variations, including the deletion of a few nucleotides in the intron region of *trn*L as compared to native species found in Australia. These genetic differences among the African *Acacia* species played a key role in the establishment of new genera, namely *Senegalia*, *Vachella*, and *Acaciella*. The presence of distinct genetic markers and variations strengthened the argument for the splitting and reclassification of *Acacia* into multiple genera, reflecting their evolutionary divergence and genetic uniqueness.

Considering such morphological and genetic characteristics, *Senegalia* was ranked as an independent genus within *Acacia* (Duarte *et al*., 2021; Sarr, 2017). The genus was reinstated to accommodate species previously assigned to *Acacia* subg. *Acculeiferum* Vass. (Seigler *et al*. 2006), as the genus *Acacia* as a whole was polyphyletic (Luckow *et al*., 2003; Miller & Seigler, 2012). Although recent molecular evidence indicates *Senegalia* is also non-monophyletic (Koenen *et al*., 2020; Ringelberg *et al*., 2022), densely sampled phylogenomic analyses are still needed before any changes to the taxonomic status of genus are made (Terra *et al*. 2022). In this context, *Senegalia* currently includes 223 species (LPWG, 2023) with prickles on branches and leaves, bipinnate leaves, extrafloral nectaries on petioles, and flowers with many free or only shortly fused stamens (Borges & Pirani, 2015; Seigler *et al*., 2017; Terra *et al*., 2022). The genus occurs throughout the global tropics and is particularly diverse in the Americas (Terra *et al*., 2022; Kyalangalilwa *et al*., 2013).

General phytochemistry of *Acacia* (or *Senegalia*) and allelopathic assays

As explained above, the *Acacia* genus was recently re-classified, therefore chemical and/or bioassay data for *Senegalia* is rare, since most species were named as *Acacia*. Due that, the research about the phytochemistry and allelochemical potential of *Acacia* species is crucial to understand the possible finds concerning *Senegalia*.

Amines, simple alkaloids, cyanogenic glycosides, cyclitols, essential oils, diterpenes, fatty acids from seed oils, fluoroacetate, gums, non-protein amino acids, triterpenes, phytosterols, saponins, flavonoids, hydrolyzable and condensed tannins have already been described for few members of the genus *Acacia* (Souza-Alonso *et al*., 2020).

Bodede *et al*. (2018) reported the phytochemical profile of *Acacia nigrescens* (= *Senegalia nigrescens* (Oliv.) P.J.H. Hurter.) the presence of various compounds. Notably, ent-kaurene diterpenoids (ent-kaur-15-en-18,20-diol and ent-kaur-15-en-18-ol) were isolated for the first time as a plants origin. Other compounds identified included 30-hydroxylup-20(29)-

en-3β-ol, 3β-hydroxy-20 (29)-en-lupan-30-al, lupeol, stigmasterol, a long-chain alcohol (tetracosanol) and three flavonoids (melanoxetin, quercetin and quercetin-3-O-methyl ether).

On the same way El Oumari *et al*. (2022) investigated the phytochemical composition of *Acacia senegal* (= *Senegalia senegal* L.) Britton) through GC-MS and confirm the presence of D-glucuronic acid, glycerol, L-grabinose, D-xylose, alpha-DL-lyxofuranoside, D-ribose, 2-methoxy(1)benzothienol(2,3-c)quinolin-6(5H)-one, palmitic acid and mandelic acid.

Castañeda-Ramírez *et al*. (2019) evaluated the phytochemical profile from the leaf extracts of *Senegalia gaumeri* (S.F. Blake) Britton & Rose through GC-MS and identified a fatty acid derivatives, like, pentacosane, heneicosane, triacontane, octacosane and hexanedioic acid bis-(2-ethylhexyl) ester. Additionally, p-coumaric acid was isolated from leaf extract using bioassay-guided purification.

Li *et al.* (2011) conducted a phytochemical investigation of *Acacia catechu* (= *Senegalia catechu* (L.f.) P.J.H.Hurter & Mabb.), revealing the presence of several compounds. The identified compounds included (3R, 4R)-3-(3,4-dihydroxyphenyl)-4-hydroxycyclohexanone, (4R)-5-(1-(3,4-dihydrophenyl)-3-oxobutyl)-dihydrofuran-2(3H)-one, 4-hydroxybenzoic acid, kaempferol, 3,4',7-trihydroxy-3',5-dimethoxyflavone, catechin, epicatechin, afzelechin, epiafzelechin, mesquitol, ophioglonin, aromadendrin and other phenol derivatives.

Recently, Cesarino *et al*. (2020) reported the leaves antioxidant and phytochemical profile of *Senegalia polyphylla* (DC.) Britton that leading to the isolation and identification of five flavonoids (luteolin, isovitexin, quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-β-D-galactopyranoside and vitexin-2”-O-rhamnoside), three triterpenes (α-amirin, β-amirin and lupeol), four steroids (stigmast-22-en-3β-ol, spinasterol, sitostanol and β-sitosterol) and an alkane (n-nonacosane).

Several studies showed that members of *Acacia* possess allelopathic activities. For example, Ismail *et al*. (2014) reported that *Acacia mangium* Willd. significantly inhibited the germination and growth of paddy varieties. Similarly, Vijayan (2015) investigated that leaves dry extract of *Acacia auriculiformis* A. Cunn. ex Benth. possess inhibitory effect on the Indian crops. Hussain *et al*. (2011) highlighted the phytotoxic effect of aqueous extracts from the flower and phyllodes of *Acacia melanoxylon* R. Br. on the *Dactylis glomerata* L., *Rumex acetosa* L., *Lolium perenne* L. and *Lactuca sativa* L., with the former presenting stronger effect in germination even at very low concentrations. The same species inhibit the growth and development of important crops including *Brassica juncea* (L.) Czern & Coss, *Phaseolus mungo* (L.), *Raphanus sativus* (L.), *Cicer arietinum* (L.), *Oryza sativa*

(L.) and *Vigna unguiculata* (L.) Walp. under lab and field conditions (Hoque *et al*., 2003; Jadhav & Gaynar, 1992). Similarly, *Acacia dealbata* (Link.) suppress the vegetative growth of *Lactuca sativa* L. var. (Reigosa *et al*., 1999).

Dissertation general goals

The main goals of this study were:

(i) investigating the phytochemical composition of leaves and stem from a Brazilian native species, *Senegalia polyphylla* (Figure 2), commonly known as a 'monjoleiro', a pioneer tree widely used in reforestation or recovery of degraded areas.

(ii) investigating the potential phytotoxic effect of leaf and stem extracts against target species.

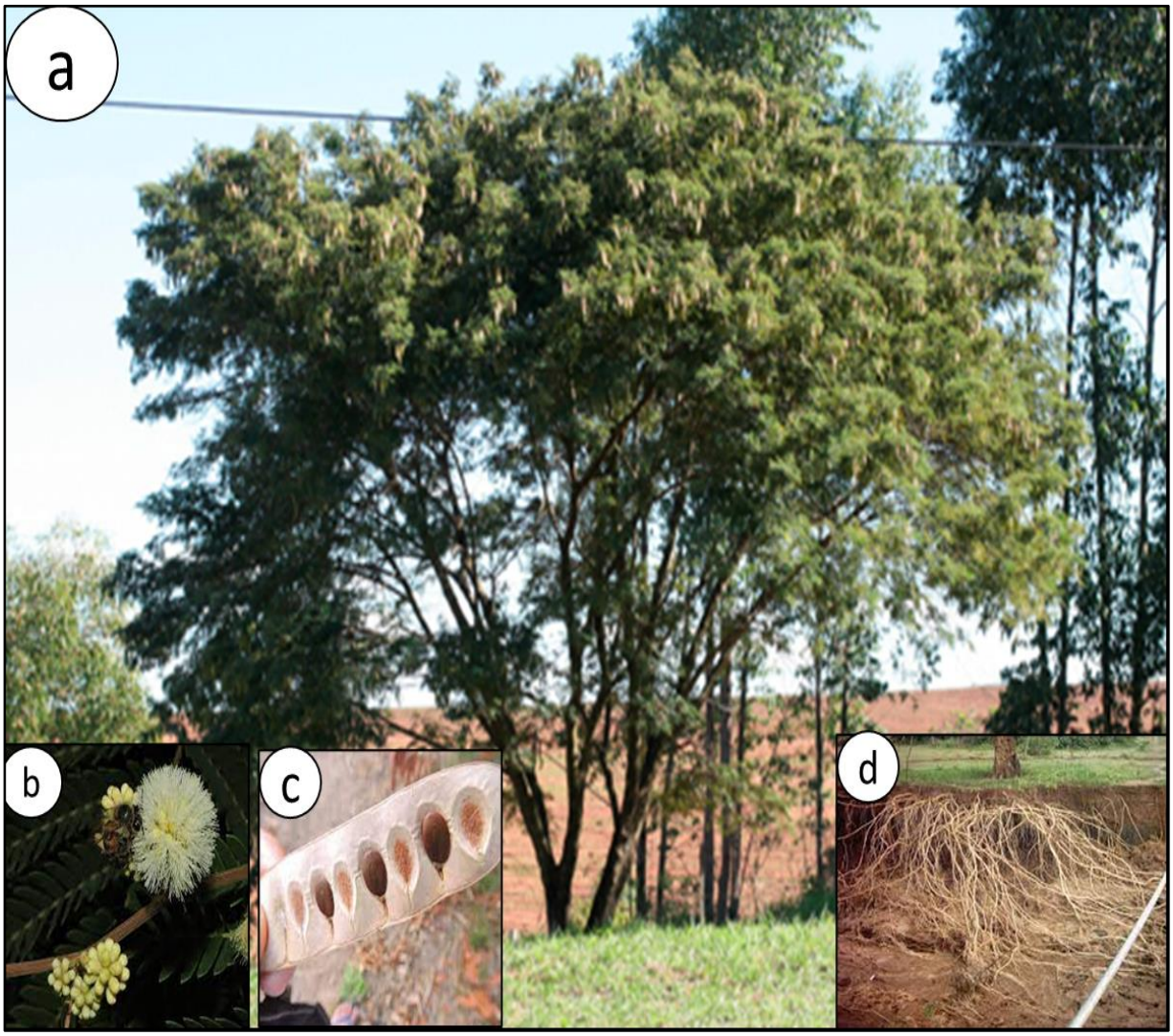


Figure 2. General view of *Senegalia polyphylla* (a), leaves and inflorescence (b), pod and seeds (c) and roots (d) (Source: Biodiversity, 2022).

References

ALHaithloul, H.A.S., Khan, M.I., Musa, A., Ghoneim, M.M., ALrashidi, A.A., Khan, I., Azab, E., Gobouri, A.A., Sofy, M.R., El-Sherbiny, M. and Soliman, M.H., 2022. Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. *PeerJ*, *10*, p.e13623.

Al-Wakeel, S.A.M., Gabr, M.A., Hamid, A.A. and Abu-El-Soud, W.M., 2007. Allelopathic effects of *Acacia nilotica* leaf residue on *Pisum sativum* L. *Allelopathy Journal*, *19*(2), p.411.

Bacellar Mendes, L.B. and Vermelho, A.B., 2013. Allelopathy as a potential strategy to improve microalgae cultivation. *Biotechnology for biofuels*, *6*(1), pp.1-14.

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M. and Vivanco, J.M., 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science*, *301*(5638), pp.1377-1380.

Barros, M.J.F. and Morim, M.P., 2014. *Senegalia* (Leguminosae, Mimosoideae) from the Atlantic Domain, Brazil. *Systematic Botany*, *39*(2), pp.452-477.

Batish, D.R., Singh, H.P., Kaur, S., Kohli, R.K. and Yadav, S.S., 2008. Caffeic acid affects early growth, and morphogenetic response of hypocotyl cuttings of mung bean (*Phaseolus aureus*). *Journal of Plant Physiology*, *165*(3), pp.297-305.

Bhowmik, P.C., 2003. Challenges and opportunities in implementing allelopathy for natural weed management. *Journal of Crops protection*, *22*(4), pp.661-671.

Bodede, O., Shaik, S. and Moodley, R., 2018. Establishment of seed germination and micropropagation protocols for *Senegalia nigrescens*, a potential anti-virulent species. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *68*(7), pp.649-655.

Borges, L.M. & Pirani, J.R. (2015) A survey of the Mimosoideae of Serra do Cipó, Minas Gerais, Brazil. In: Fortunato, R. (ed.) V *Conferencia Internacional de Leguminosas*, VILC, 41-56.

Castañeda-Ramírez, G.S., de Jesús Torres-Acosta, J.F., Sandoval-Castro, C.A., Borges-Argáez, R., Cáceres-Farfán, M., Mancilla-Montelongo, G. and Mathieu, C., 2019. Bio-guided fractionation to identify *Senegalia gaumeri* leaf extract compounds with anthelmintic

activity against *Haemonchus contortus* eggs and larvae. *Veterinary Parasitology*, *270*, pp.13-19.

Cesarino, Elaine Caçal. "Contribuição ao estudo químico de *Acacia Polyphylla* e avaliação da atividade antioxidante." (2019).

Cheng, F. and Cheng, Z., 2015. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in plant science*, *6*, p.1020.

de Albuquerque, M. B., dos Santos, R. C., Lima, L. M., Melo Filho, P. D. A., Nogueira, R. J. M. C., Da Câmara, C. A. G., & de Rezende Ramos, A. (2011). Allelopathy, an alternative tool to improve cropping systems. A review. *Agronomy for Sustainable Development*, *31*, 379-395.

Dirks, I., Raviv, B., Shelef, O., Hill, A., Eppel, A., Aidoo, M. K., ... & Rachmilevitch, S. (2016). Green roofs: what can we learn from desert plants?. *Israel Journal of Ecology & Evolution*, *62*(1-2), 58-67.

Duarte, A.F.T., Ribeiro, P.G., Moreira, A.L.D.C., de Queiroz, L.P. and de Assis Ribeiro dos Santos, F., 2021. Pollen morphology of *Senegalia* Raf. species and related genera (Leguminosae: Caesalpinioideae: mimosoid clade). *Palynology*, *45*(4), pp.627-639.

Duke, S. O. (2015). Proving allelopathy in crop–weed interactions. *Weed Science*, *63*(SP1), 121-132.

El Oumari, F.E., Bousta, D., Imtara, H., Lahrichi, A., Elhabbani, R., El Mouhri, G., Al Kamaly, O., Saleh, A., Parvez, M.K., Grafov, A. and Sqalli Houssaini, T., 2022. Chemical Composition and Anti-Urolithiatic Activity of Extracts from *Argania spinosa* (L.) Skeels Press-Cake and *Acacia senegal* (L.) Willd. *Molecules*, *27*(13), p.3973.

Farhoudi, R. and Lee, D.J., 2013. Allelopathic effects of barley extract (Hordeum vulgare) on sucrose synthase activity, lipid peroxidation and antioxidant enzymatic activities of *Hordeum spontoneum* and *Avena ludoviciana*. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *83*, pp.447-452.

Gebrekiros, S. T. (2016). Factors affecting stream fish community composition and habitat suitability. *Journal of Aquaculture & Marine Biology*, *4*(2), 00076.

Ghori, N.H., Ghori, T., Hayat, M.Q., Imadi, S.R., Gul, A., Altay, V. and Ozturk, M., 2019. Heavy metal stress and responses in plants. *International journal of environmental science and technology*, *16*, pp.1807-1828.

Gupta, D.K., Palma, J.M. and Corpas, F.J. eds., 2018. *Antioxidants and antioxidant enzymes in higher plants*. Berlin: Springer International Publishing.

Hassan, M. S., Naz, N., Ali, H., Ali, B., Akram, M., Iqbal, R., ... & Hassan, Z. (2023). Ultra-Responses of *Asphodelus tenuifolius* L.(Wild Onion) and *Convolvulus arvensis* L.(Field Bindweed) against Shoot Extract of *Trianthema portulacastrum* L.(Horse Purslane). *Plants*, *12*(3), 458.

Hoque, A.T.M.R., Ahmed, R., Uddin, M. and Hossain, M., 2003. Allelopathic effect of different concentration of water extracts of *Acacia auriculiformis* leaf on some initial growth parameters of five common agricultural crops. *Pakistan Journal of Agronomy*, *2*(2), pp.92-100.

Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H. and Yu, J.Q., 2013. Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. *Journal of chemical ecology*, *39*, pp.232-242.

Hussain, F., Ahmad, B., & Ilahi, I. (2010). Allelopathic effects of *Cenchrus ciliaris* L. and *Bothriochloa pertusa* (L.) A. Camus. *Pakistan Journal of Botany*, *42*(5), 3587-3604.

Hussain, M. I., Gonzalez, L., & Reigosa, M. J. (2011). Allelopathic potential of *Acacia melanoxylon* on the germination and root growth of native species. *Weed Biology and Management*, *11*(1), 18-28.

Ighodaro, O.M. and Akinloye, O.A., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. *Alexandria journal of medicine*, *54*(4), pp.287-293.

Ismail, N. A. N., & Metali, F. (2014). Allelopathic effects of invasive *Acacia mangium* on germination and growth of local paddy varieties. *Journal of Agronomy*, *13*(4), 158-168.

Jabran, K. and Jabran, K., 2017. Allelopathy: Introduction and concepts. *Manipulation of allelopathic crops for weed control*, pp.1-12.

Jadhav, B. B., & Gaynar, D. G. (1992). Allelopathic effects of *Acacia auriculiformis* A. Cunn. on germination of rice and cowpea. *Indian Journal of Plant Physiology*, *35*, 86-86.

John, J. and Sarada, S., 2012. Role of phenolics in allelopathic interactions. *Allelopathy Journal*, *29*(2).

Justen, V.L. and Fritz, V.A., 2013. Temperature-induced glucosinolate accumulation is associated with expression of BrMYB transcription factors. *Hortscience*, *48*(1), pp.47-52.

Karageorgou, P., Levizou, E. and Manetas, Y., 2002. The influence of drought, shade and availability of mineral nutrients on exudate phenolics of *Dittrichia viscosa*. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *197*(4), pp.285-289.

Kensa, V.M., 2011. Bioremediation-an overview. *Journal of Industrial Pollution Control*, *27*(2), pp.161-168.

Khalil, S.K., Mehmood, T., Rehman, A., Wahab, S., Khan, A.Z., Zubair, M., Mohammad, F., Khan, N.U. and Khalil, I.H., 2010. Utilization of allelopathy and planting geometry for weed management and dry matter production of maize. *Pakistan Journal of Botany*, *42*(2), pp.791-803.

Kopsell, D.A. and Kopsell, D.E., 2006. Accumulation and bioavailability of dietary carotenoids in vegetable crops. *Trends in plant science*, *11*(10), pp.499-507.

Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021). An overview of some biopesticides and their importance in plant protection for commercial acceptance. *Plants*, *10*(6), 1185.

Kumar, S., Sharma, A. K., Rawat, S. S., Jain, D. K., & Ghosh, S. (2013). Use of pesticides in agriculture and livestock animals and its impact on environment of India. *Asian Journal of Environmental Science*, *8*(1), 51-57.

Kyalangalilwa, B., Boatwright, J. S., Daru, B. H., Maurin, O., & van der Bank, M. (2013). Phylogenetic position and revised classification of *Acacia sl* (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. *Botanical Journal of the Linnean Society*, *172*(4), 500-523.

Li, B., Vik, S.B. and Tu, Y., 2012. Theaflavins inhibit the ATP synthase and the respiratory chain without increasing superoxide production. *The Journal of Nutritional Biochemistry*, *23*(8), pp.953-960.

Li, X.C., Liu, C., Yang, L.X. and Chen, R.Y., 2011. Phenolic compounds from the aqueous extract of *Acacia catechu*. *Journal of Asian natural products research*, *13*(9), pp.826-830.

Li, Z. R., Amist, N., & Bai, L. Y. (2019). Allelopathy in sustainable weeds management. *Allelopathy J*, *48*, 109-138.

Liebman, M., & Davis, A. S. (2000). Integration of soil, crop and weed management in low-external-input farming systems. *WEED RESEARCH-OXFORD-*, *40*(1), 27-48.

LPWG (2023). The World Checklist of Vascular Plants (WCVP): Fabaceae (R. Govaerts, Ed.; 2023v.3). Royal Botanic Gardens, Kew. https://doi.org/10.15468/mvhaj3

Luckow, M., Miller, J. T., Murphy, D. J., & Livshultz, T. A. T. Y. A. N. A. (2003). A phylogenetic analysis of the Mimosoideae (Leguminosae) based on chloroplast DNA sequence data. Advances in legume systematics, part, 10, 197-220.

Lundkvist, A., & Verwijst, T. (2011). Weed biology and weed management in organic farming. *Research in Organic Farming*, 10-41.

Macias, F.A., Molinillo, J.M., Varela, R.M. and Galindo, J.C., 2007. Allelopathy—a natural alternative for weed control. *Pest Management Science: Formerly Pesticide Science*, *63*(4), pp.327-348.

Meiners, S.J., Kong, C.H., Ladwig, L.M., Pisula, N.L. and Lang, K.A., 2012. Developing an ecological context for allelopathy. *Plant Ecology*, *213*, pp.1221-1227.

Meksawat, S. and Pornprom, T., 2010. Allelopathic effect of itchgrass (*Rottboellia cochinchinensis*) on seed germination and plant growth. *Weed Biology and management*, *10*(1), pp.16-24.

Miller, J. T., & Seigler, D. (2012). Evolutionary and taxonomic relationships of *Acacia sl* (Leguminosae: Mimosoideae). *Australian Systematic Botany*, 25(3), 217-224.

Molisch, H., 1937. Der Einfluss einer Pflanze auf die andere, Allelopathie. *Fischer Jena*.

Mølmann, J.A., Steindal, A.L., Bengtsson, G.B., Seljåsen, R., Lea, P., Skaret, J. and Johansen, T.J., 2015. Effects of temperature and photoperiod on sensory quality and contents of glucosinolates, flavonols and vitamin C in broccoli florets. *Food chemistry*, *172*, pp.47-55.

Muhammad, Z. and Majeed, A.B.D.U.L., 2014. Allelopathic effects of aqueous extracts of sunflower on wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). *Pakistan Journal of Botany*, *46*(5), pp.1715-1718.

Otusanya, O. and Ilori, O., 2012. Phytochemical screening and the phytotoxic effects of aqueous extracts of *Tithonia diversifolia* (Hemsl) a. Gray. *International Journal of Biology*, *4*(3), p.97.

Putnam, A.R., 1985. Weed allelopathy. *Weed physiology*, *1*, pp.131-155.

Reigosa, M., Pedrol, N. and Gonzlez, L., 2002. *Allelopathy* (pp. 209-226). Science Publishers, Inc.

Rice, E.L., 1984. Manipulated ecosystems: Roles of allelopathy in agriculture. *Allelopathy. Academic Press, New York*, pp.1-422.

Ringelberg, J. J., Koenen, E. J., Iganci, J. R., de Queiroz, L. P., Murphy, D. J., Gaudeul, M., ... & Hughes, C. E. (2022). Phylogenomic analysis of 997 nuclear genes reveals the need for extensive generic re-delimitation in Caesalpinioideae (Leguminosae). *PhytoKeys*, 205, 3.

Rizvi, S. J. H., Tahir, M., Rizvi, V., Kohli, R. K., & Ansari, A. (1999). Allelopathic interactions in agroforestry systems. *Critical reviews in plant sciences*, *18*(6), 773-796.

Sarr, M.S., 2017. *Morphological and Physiological Responses of Senegalia senegal (L.) Britton Provenances to Drought, Salinity, and Fertility* (Doctoral dissertation, Virginia Tech).

Seigler, D. S., Ebinger, J. E., & Miller, J. T. (2006). The genus *Senegalia* (Fabaceae: Mimosoideae) from the new world. *Phytologia*, 88(1), 38-96.

Seigler, D. S., Ebinger, J. E., Riggins, C. W., Terra, V., & Miller, J. T. (2017). *Parasenegalia* and *Pseudosenegalia* (Fabaceae): new genera of the Mimosoideae. Novon: *A Journal for Botanical Nomenclature*, 25(2), 180-205.

Seigler, D.S., Ebinger, J.E. and Miller, J.T., 2006. Mariosousa, a new segregate genus from *Acacia sl* (Fabaceae, Mimosoideae) from Central and North America. *Novon: A Journal for Botanical Nomenclature*, *16*(3), pp.413-420.

Souza-Alonso, P., Puig, C.G., Pedrol, N., Freitas, H., Rodríguez-Echeverría, S. and Lorenzo, P., 2020. Exploring the use of residues from the invasive *Acacia* sp. for weed control. *Renewable Agriculture and Food Systems*, *35*(1), pp.26-37.

Sverdlov, A.L., Elezaby, A., Behring, J.B., Bachschmid, M.M., Luptak, I., Tu, V.H., Siwik, D.A., Miller, E.J., Liesa, M., Shirihai, O.S. and Pimentel, D.R., 2015. High fat, high sucrose diet causes cardiac mitochondrial dysfunction due in part to oxidative post-translational modification of mitochondrial complex II. *Journal of molecular and cellular cardiology*, *78*, pp.165-173.

Taiz, L. and Zeiger, E., 2010. Responses and adaptations to abiotic stress. *Plant Physiology, Fifth Edition. Sunderland, MA: Sinauer Associates, Inc*, pp.755-778.

Terra, V., Ringelberg, J. J., Maslin, B., Koenen, E. J., Ebinger, J., Seigler, D., & Hughes, C. E. (2022). Dilemmas in generic delimitation of *Senegalia* and allies (Caesalpinioideae, mimosoid clade): how to reconcile phylogenomic evidence with morphology and taxonomy?. *PhytoKeys*, 205, 261.

Turk, M.A., Lee, K.D. and Tawaha, A.M., 2005. Inhibitory effects of aqueous extracts of black mustard on germination and growth of radish. *Research Journal of Agriculture and Biological. Science*, *1*(3), pp.227-231.

Vijayan, V. (2015). Evaluation for allelopathic impact of *Acacia auriculiformis* A. Cunn. ex Benth on Seed germination and Seedling growth of Rice (*Oryza sativa* L), a widely cultivated Indian crop species. *Research Journal of Agriculture and Forestry Sciences ISSN*, *2320*, 6063.

Wardle, D. A., Karban, R., & Callaway, R. M. (2011). The ecosystem and evolutionary contexts of allelopathy. *Trends in ecology & evolution*, *26*(12), 655-662.

Weir, T. L., Park, S. W., & Vivanco, J. M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current opinion in plant biology*, *7*(4), 472-479.

Weston, L. A. (2003). Root exudates: an overview. *Root ecology*, 235-255.

Zeng, R.S., Luo, S.M., Shi, Y.H., Shi, M.B. and Tu, C.Y., 2001. Physiological and biochemical mechanism of allelopathy of secalonic acid F on higher plants. *Agronomy journal*, *93*(1), pp.72-79.

Zhou, Y.H. and Yu, J.Q., 2006. Allelochemicals and photosynthesis. In *Allelopathy: A physiological process with ecological implications* (pp. 127-139). Dordrecht: *Springer Netherlands*.

Zuo, S., Zuo, Y., Gu, W., Wei, S. and Li, J., 2022. Exogenous Proline Optimizes Osmotic Adjustment Substances and Active Oxygen Metabolism of Maize Embryo under Low-Temperature Stress and Metabolomic Analysis. *Processes*, *10*(7), p.1388.

CHAPTER 1

Chemical profile and phytotoxic effect of *Senegalia polyphylla* (DC.) Britton stem crude extract and partition phases on target species

Abstract

Allelopathy is an emerging science with potential applications in agriculture, horticulture, botany, and forestry. This study aimed to investigate the chemical profile of the stem of *Senegalia polyphylla* and its effects on wheat coleoptile elongation and initial growth on target species. Dried stem fragments were powdered and extracted with 70% ethanol, and further partitioned with increasing polarity solvents. The stem crude extract (CE) and its partition phases were evaluated against wheat coleoptile elongation, and germination and initial growth of target plants in three distinct concentrations (0.2, 0.4 and 0.8 $mg.mL^{-1}$). Morphological (fresh mass, root and shoot growth, root anatomy) and biochemical (photosynthetic pigments and antioxidant enzymes) parameters were measured in target plants. Chemical composition of CE and partition phases was analyzed by GC-MS, HPLC-DAD and HPLC-MS/MS. Mass spectra were used for compounds annotation through comparison with data bases (NYST library and GNPS) and literature. Although all samples, except ethyl acetate partition phase, significantly affected coleoptile elongation, at all concentrations, only the butanol (BuOH) and Hx/DCM partition phases significantly affected the germination rate, root and shoot length, and fresh biomass of lettuce and tomato seedlings. Generally, Hx/DCM caused more pronounced effect than BuOH, and tomato was more sensitive than lettuce. Furthermore, these partition phases lead to a decrease in the photosynthetic pigments (chlorophyll *a*, *b* and carotenoids), and to an upregulation of SOD activity in the target species, while, CAT and APX were differentially affected in tomato and lettuce seedlings. Aerenchyma tissues, smaller radial cortical cells and intercellular spaces were observed in the roots of the target species Based on GNPS annotation, HPLC-MS/MS analysis revealed the presence of 21 compounds, including isoflavone, flavonol and flavone glycosides, and flavone derivatives, phospholipids, glycolipids, glyceride and alkaloid derivatives. Similarly, GC-MS analysis of hexane/DCM partition phases detected the presence of n-hexadecanoic acid, squalene, chondrillasterol, lup-20(29)-3-en-3one and lupeol derivatives. Further research is recommended to unrevealed potential active compounds and its effect on important crops in field condition.

Key words; extraction, GNPS, coleoptile bioassay, HPLC-MS/MS, allelochemicals, target species

1.1 Introduction

Biological and chemical interactions among organisms are the essential determinants of the communities' habitat (Gebrekiros, 2016). These interactions may be merely for abiotic requirements like space, light or energy, water and nutrients, including nitrogen, potassium and phosphorus etc. (Geider *et al*., 2001; Moore *et al*., 2013). Similarly, plants also require such resources to interact with one another *via* plant-plant allelopathic interaction and plant–plant resource competition (Blum & Blum, 2011).

Rice (1968) defined allelopathy as a phenomenon in which donor plants affect (negatively or/and positively) the germination, growth and development of other acceptor–plants, mediated by the addition of plant-produced secondary products (allelochemicals). These compounds are present in all plant's tissues including fruits, buds, bark, stem, flowers, roots, leaves, rhizomes, pollen, seeds and soil leachates (Rewald *et al*., 2018; Li *et al*., 2022) and can be released by different ways like, volatilization, leaching, decomposition of residues and root exudation (Zohaib *et al*., 2016).

The phytochemical profile and allelopathic potential of the *Acacia* genus have been studied by many research groups (Jelassi *et al*., 2016; Hussain *et al*., 2020; Abdel-Farid *et al*., 2014). Molecular and morphological studies recently proposed the segregation of some species of *Acacia* to *Senegalia* (Miller & Seigler, 2012). Due that, most allelopathic and phytochemical data available are related to the former genus.

Amines, simple alkaloids, cyanogenic glycosides, cyclitols, essential oils, diterpenes, fatty acids from seed oils, fluoroacetate, gums, non-protein amino acids, triterpenes, phytosterols, saponins, flavonoids, hydrolysable and condensed tannins have already been described for few members of the genus *Acacia* (Souza-Alonso *et al*., 2020).

Reigosa & Carballeira (2016) demonstrated that leachate from *Acacia dealbata* Link. exhibited a strong inhibitory effect on the germination and growth of *Lactuca sativa* L. *in vitro*. Additionally, Wakeel *et al*. (2007) investigated the allelopathic effects of *Acacia nilotica* (L.) Willd stem bark and leaves on various crop species and found ferulic acid, caffeic acid, cinamic acid, apigenin and tannins to be major components that inhibit the growth and development of tomatoes. In a similar context, Chou *et al*. (1998) studied the allelopathic effect of *Acacia confusa* Merr (Britton & Rose) and highlighted its effectiveness, even at a lower concentration of 0.5%, against *Raphanus acanthiformis* L, *Lactuca sativa* L, *Brassica chinensis* L., and *Medicag sativa* L. Various phytochemicals, including vanillic acid, gallic acid, ferulic acid, m-hydroxybenzoic acid, caffeic acid, m-hydroxyphenylactic acid, and unidentified flavonoids, were characterized from the aqueous extract and ether partition. Hussain *et al*. (2011)

highlighted the germination inhibition of aqueous extracts from the flower and phyllodes of *Acacia melanoxylon* R. Br. on the *Dactylis glomerata* L., *Rumex acetosa* L., *Lolium perenne* L. and *Lactuca sativa* L. The same species also inhibited the growth and development of *Brassica juncea* (L.) Czern & Coss; *Phaseolus mungo* (L.); *Raphanus sativus* (L.); *Vigna unguiculata* (L.) Walp., *Cicer arietinum* (L.), *Oryza sativa* (L.) and *Vigna unguiculata* (L.) Walp. (Hoque *et al*., 2003; Jadhav & Gaynar, 1992).

The genus *Senegalia* (Fabaceae) comprises approximately 220 species (LPWG, 2023), many of which are abundant in phenolic compounds known for their antioxidant potential in scavenging reactive oxygen species (ROS) and inhibiting the generation of free radicals (Scabora *et al*., 2011). *Senegalia polyphylla* (DC.) Britton and Rose, commonly referred to as 'monjoleiro,' is a native Brazilian plant found in diverse regions including the Amazon, Caatinga, Cerrado, Atlantic Forest and Pantanal (Barros *et al*., 2014). Traditionally, its resin has been used to alleviate severe coughs, while its seeds have shown insecticidal activity against *Anagasta kuehniella* (Machado *et al*., 2013). Cesarino *et al*. (2020) reported the phytochemical profile and the antioxidant potential of the leaves of *Senegalia polyphylla*, thereby, describing five flavonoids (luteolin, isovitexin, quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-β-D-galactopyranoside and vitexin-2"-O-rhamnoside), three triterpenes (α-amirin, β-amirin, and lupeol), four steroids (stigmast-22-en-3β-ol, spinasterol, sitostanol, and β-sitosterol) and an alkane (n-nonacosane). However, this is the only source of information regarding the chemical and biological activities of this species. Hence, the present study aims to investigate the chemical profile of the stem of *S. polyphylla*, as well as the effects of the crude extract and partition phases on the germination, initial growth, biochemical parameters and anatomical changes in target plants such as lettuce and tomato.

1.2 Material and method

1.2.1 Plants collection

Senegalia polyphylla (DC.) Britton and Rose branches were collected in the natural habitat of São Carlos, São Paulo, Brazil, in May 2022. The species was properly identified by Prof. Dr Leonardo Borges (Federal University of São Carlos – UFSCar), and a voucher material (LM Borges 1253) is available at SPSC Herbarium.

1.2.2 Crude extract and partition phases preparation

The samples were kept in the oven (at 60 ^{o}C for at least 2 days). The stems were separated and grinded. 200 g of powder dried stem was macerated in 70% ethanol (1 g/25mL)

for seven days, with three total solvent replacements (Novaes *et al*., 2016). The extracts were then pooled, filtered, and concentrated in a rotary evaporator for the crude extract (CE – 20.8 g).

An aliquot of CE (15 g) was further dissolved in 30% ethanol and partitioned with 250 mL of solvents of increasing polarities, i.e. hexane (Hx), dichloromethane (DCM), ethyl-acetate (EtOAc), and butanol (BuOH), yielding five partition phases: Hx (0.38 g), DCM (0.62 g), BuOH (1.7 g), EtOAc (0.39 g), and the hydroalcoholic residue (EtOH – 11.29 g).

1.2.3 High performance liquid chromatography (HPLC) analysis

CE and the polar partition phases (BuOH, EtOAc, and EtOH) were analyzed by HPLC coupled to a diode array detector (DAD) (Agilent 1200). Chromatographic separation was carried out using a reverse phase Zorbax Eclipse plus C_{18} column (3.5µ-150.0mm x 4.6mm) and a mobile phase gradient consisting of 0.1% acetic acid (AcOH) and acetonitrile (CH_3CN). The chromatographic conditions included an initial CH_3CN concentration of 10% (0-6 min), followed by an increase to 15% (6-7 min). This concentration was maintained for 15 minutes, after which it was raised to 50% (22-32 min) and subsequently to 100% (32-42 min), remaining constant for an additional 8 minutes. Detection was performed at specific wavelengths, including 352 nm, 325 nm, 300 nm, and 280 nm. The column was at 45^{o}C, and each peak was scanned from λ = 200 – 600 nm.

The crude extract was also analyzed by HPLC coupled to high resolution mass spectrometer (HPLC-MS-MS) (Shimadzu Nexera X_2 - MicroTOF-QII). The chromatographic conditions were the same described above. The mass spectrometer equipped with and electrospray (ESI) operated under positive mode at mass range of *m/z* 50-1200. The positive ionization on ESI was fixed as: capillarity voltage of 4500 V and end plate offset at 500 V. Nitrogen (N_2) was used as dry gas at flow rate of 0.8 mL.min^{-1}, with pressure level of 4.0 Bar and 200 ^{o}C temperature. Additionally, the collision-induced dissociation (CID) energy was properly set at 25 eV.

1.2.4 Gas-chromatography coupled to mass spectrometry (GC/MS) analysis

The analysis of Hx and DCM partition phases was carried out in an Agilent system (Agilent 6850/5975) equipped with a DB-5-HT capillary column (30 m x 0.32nm x 0.25 id). Helium was used as a carrier gas with 1.5 mL.min^{-1} flux. The column temperature was kept at 50 °C for 2 minutes and then increased progressively up to 350 °C at 3 °C.min^{-1}, while the

temperature of injector was at 300 ºC. Additionally, the mass spectra were acquired using electronic ionization at 70 eV in the full-scan mode, with mass range between 40 to 700 m/z and 2.66 scans.s^{-1}. The MS source and quadrupole temperatures were 250 °C and 200 °C respectively. The relative proportion of constituents were calculated by the areas of the respective peaks.

1.2.5 Molecular Networking

The molecular network was generated by using online workflow (https://ccms-ucsd.github.io/GNPSDocumentation/) on GNPS website (http://gnps.ucsd.edu). The data was filtered with complete removal of MS/MS fragment ions within range of +/- 17 Da of the precursor *m/z*. Similarly, the MS/MS spectra were also window filtered by selecting only the top 6 fragment ions in the +/- 50 Da window throughout the spectrum, respectively. The precursor ion mass tolerance was set to 2.0 Da and a MS/MS fragment ion tolerance of 0.5 Da. A network was then created where edges were filtered to have a cosine score above 0.7 and more than 4 matched peaks. Furthermore, the edges between two nodes were kept in the network if and only if each of the nodes appeared in each other's respective top 10 most similar nodes. Finally, the maximum size of a molecular family was set to 100 and the lowest scoring edges were removed from molecular families until the molecular family size was below this threshold. The spectra in the network were then searched against GNPS' spectral libraries. The library spectra were filtered in the same manner as the input data. All matches kept between network spectra and library spectra were required to have a score above 0.7 and at least 4 matched peaks.

1.2.6 Coleoptile bioassay

Healthy wheat seeds (*Triticum aestivum* L.) were sown in petri dishes lined with autoclaved Whatman filter paper, moistened with distilled water. The petri dishes were covered with aluminum foil and placed into a BOD at 25 °C for 4 days. After this period, the petri dishes were unfoiled under green light and the etiolated seedlings used for the assay. The upper 2 mm of the coleoptile was cut and discarded, while the next 4mm was also cut and selected for bioassay (Rial *et al*., 2014).

CE and partition phases were diluted in three different concentrations (0.8, 0.4 and 0.2 $mg.mL^{-1}$) using phosphate-citrate buffer solution consisting of 2% sucrose and 0.5% DMSO (dimethyl sulfo-oxide) with 5.6 pH. Additionally, negative control was represented by buffer only and commercial herbicide (glyphosate) was used as a positive control in the same

concentrations of the samples. Five 4 mm-coleoptile fragments and 2 mL of each extract and controls were transferred into test tubes. Each treatment and controls were prepared in triplicates. All tubes were placed in rotator and kept in BOD under dark condition with 25 °C. After 24 hours, incubated fragments were measured using ImageJ software (version 1.54d). Length parameters are presented as percentage differences from their respective control.

1.2.7 Germination and seedling initial growth bioassay

Healthy diaspores of *Solanum lycopersicum* Mill. (tomato) and *Lactuca sativa* L. (lettuce) were selected for the germination bioassay. Six-wells microplates were lined with Whatman N.1 filter paper and kept 10 diapores in each well. Each well received 1 mL of CE, Hx, BuOH and EtOH partition phases prepared with distilled water in the same concentrations used for coleoptile assay. Positive control was also made with glyphosate, with same concentrations described before, and negative control with distilled water. Samples were analyzed with five replicates for each treatment. The plates were sealed with parafilm, labeled and incubated in the growth chamber with 25 °C and 12 h light. After seven days of incubation, different growth traits i.e. germination rate and root and shoot length were measured using ImageJ software (Version 1.54d) when needed. On the same way, fresh biomass was also monitored, using analytical balance. The data are presented as percentage difference from test samples to negative control.

1.2.8 Anatomical analysis

After germination assay, 2–5 seedlings from Hx/DCM and BuOH partition phases treatments, besides negative control, were fixed with Karnovsky reagent (Karnovsky, 1965). The seedlings were dehydrated in an ethyl alcohol series and embedded in historesin (Johansen, 1940). Transverse and longitudinal serial sections with 10 µm thickness were made using Leica RM2145 rotary microtome. The sections were stained with 0.05% toluidine blue in 0.1 M sodium acetate buffer pH 4.7 and mounted in Permount resin (O'Brien *et al*., 1964). Finally, the images were taken with a Leica DMLB microscope equipped with a Leica DFC 310FX digital camera.

1.2.9 Pigment content determination

Chlorophyll content (*a*, *b*) and carotenoids were determined in the target species submitted to Hx/DCM and BuOH partition phases and negative control. Seedling leaves (50 mg) were transferred to a 2 mL Eppendorf and frozen at -80 ^{o}C until the analysis. Frozen

samples were grinded in liquid nitrogen (N_2), added with 2 mL of methanol, and centrifuged at 14, 000 rpm for 5 minutes, at 4 °C. Supernatants (90 µL) were taken, transferred to 96-wells microplates, and the optical density (UV) were determined at 448, 453 and 476 nm using ELISA reader (Torres *et al*., 2014 with modifications). All readings were done in triplicate. For quantification following formulas were used,

Chlorophyll *a* = $12.6 \times \text{Abs } 666$

Chlorophyll *b* = $34.09 \times \text{Abs } 649 - 15.28 \times \text{Abs } 666$

Carotenoids = $1000 \times \text{Abs } 470 - 1.63 \times \text{Chl } a / 221$

Where, Abs = absorbance, chl *a* = chlorophyll a and final concentration was expressed in $\mu g.mL^{-1}$.

1.2.10 Antioxidant enzymes activities

The protein extraction and the activity of some antioxidant enzymes in target seedlings exposed to Hx/DCM and BuOH partition phases and negative control were realized following adaptations done by Sala-Carvalho *et al*. (2022).

A total of 200 mg frozen samples from each treatment, i.e. 0.2, 0.4 and 0.8 $mg.mL^{-1}$, were grinded in dark using mortar and pistil, helped with liquid N_2. 1.5 mL of extraction buffer (20 mL of 50 mM potassium phosphate buffer (pH 7.8), 17.6 mg of ascorbic acid and 15.42 µL of dichlorodiphenyltrichloroethane) and 30 mg of PVPP were added and centrifugation was carried out at 10,000 rpm for 10 min at 4 °C. Total soluble proteins were determined using Bradford reagent for the supernatant and bovine serum albumin as a standard. Using 96-wells microplate, 40 µL of extracts (in triplicate) and 200 µL of Bradford's reagent were added to each well. The plate was stirred for 1 min and absorbance was checked at 590 nm using ELISA reader Ultrospec™ 7000.

Three supernatant aliquots of 200 µL were collected and stored at -80 °C for further analysis of superoxide dismutase (SOD) (EC 1.15.1.1), ascorbate peroxidase (APX) (EC 1.11.1.1) and catalase (CA) (EC 1.11.1.6).

The SOD activity was determined based on potential inhibition of photo-reduction of nitro tetrazolium blue (NBT). Into wells of two 96-wells microplates, 40 µL of EDTA, followed by 40 µL of methionine, 80 µL of 100 mM potassium phosphate buffer, 40 µL NBT and 16 µL riboflavin were added. Right away, reaction was started by adding 40 µL of enzyme extracts into each well. Then, one plate was rest in dark for 30 min and the other were kept in light. For

negative control, the reaction mixture was used. After rest, the absorbance was measured at 560 nm. All reactions were done in triplicates. The SOD activity was determined by subtracting dark-plate absorbance values from respective light-plate values. The calculated SOD contents were expressed in $U.mg^{-1}$ of protein.

Catalase (CAT) was determined based on the oxidation of H_2O_2. Using 96-wells microplate, 20 µL of prepared sample were taken in triplicates and added with 170 µL of 100 mM potassium phosphate buffer with pH 7.5. Next, 10 µL of 200 mM H_2O_2 were also added to the sample mixture. For negative control, 20 µL of 100 mM potassium phosphate buffer (pH 7.5) replace the extract. Absorbance at 240 nm was checked before and after the addition of H_2O_2 (every 30 seconds for 2 minutes), at 25 ^oC and was expressed in µmol H_2O_2. min^{-1}. mg^{-1} of protein.

Ascorbate peroxidase (APX) activity was determined by assessing the ascorbate oxidation rate. Aliquots of 24 µL of sample extracts were transferred into a well of 96-wells microplates, in triplicates. Further, 146 µL of APX reaction buffer (14.4 mL of 100 mM potassium phosphate buffer pH 7.0 and 3.4 mL of EDTA) and 20 µL of 5 mM ascorbate were added. Then, 10 µL of H_2O_2 were also added to the reaction mixture. For negative control, the extract volume was replaced by APX reaction. The absorbance was checked at 290 nm, before adding H_2O_2 and after to every 30s for 2 minutes at 30 °C and were expressed in µmol H_2O_2. min^{-1}. mg^{-1} of protein**.**

1.2.11 Statistical analysis

Data were recorded in the form of biological triplicates for the coleoptile bioassay. Similarly, seed germination and fresh biomass bioassay data were recorded from the five biological replicates, while for shoot and root length the biological replicates were dependent on number of germinated seedlings. Measurement was taken using ImageJ software (version 7.0). On the same way, data was statistically assessed by one-way analysis of variance (ANOVA) using SPSS v. 21.0 software package. Mean separations were performed by Post hoc Duncan's multiple range tests. Differences at $p < 0.05$ were considered significant, using asterisks.

1.3 Results

1.3.1 Phytochemical composition of stem of Senegalia polyphylla

Through GNPS analysis, 21 compounds were annotated in the stem crude extract of *Senegalia polyphylla*. These annotated compounds were grouped as lipids, flavonoids, alkaloid and terpenoid derivative (Table 1, Figure 1).

Different flavonoids annotated are isoflavones (3'-methoxypuerarin and 3'-hydroxypuerarin), flavonol glycosides (herbacetin-3, 8-diglucopyranoside, luteolin-6-C-glucoside, rutin, kaempferol 3-O-rutinoside and pectolinarin), flavone glycoside (2"-O-beta-L-galactopyranosylorientin) and flavone derivatives (luteolin and 6,4'-Dimethoxy-3-hydroxyflavone). Similarly, one pyrrolizidine alkaloid i.e. senecionine was also annotated. On the same way, different lipids derivatives annotated are phospholipids (unidentified phosphatidylcholine derivatives), glycolipids (monogalactosyldiacylglycerol and digalactosyldiacylglycerol) and a glyceride (diacylglycerol). These compounds distribution in the polar partition phases was done based on comparison of the retention time among chromatograms (Table 1).

GC-MS analysis of apolar partition phases hexane (Hx) and dichloromethane (DCM) revealed the presence of five compounds based on NIST suggestions and confirmations from literature (Table 2). These compounds belong to fatty acid (n-hexadecanoic acid), sterols (chondrillasterol) and triterpenes derivatives (squalene, lup-20(29)-3-en-3one and lupeol).

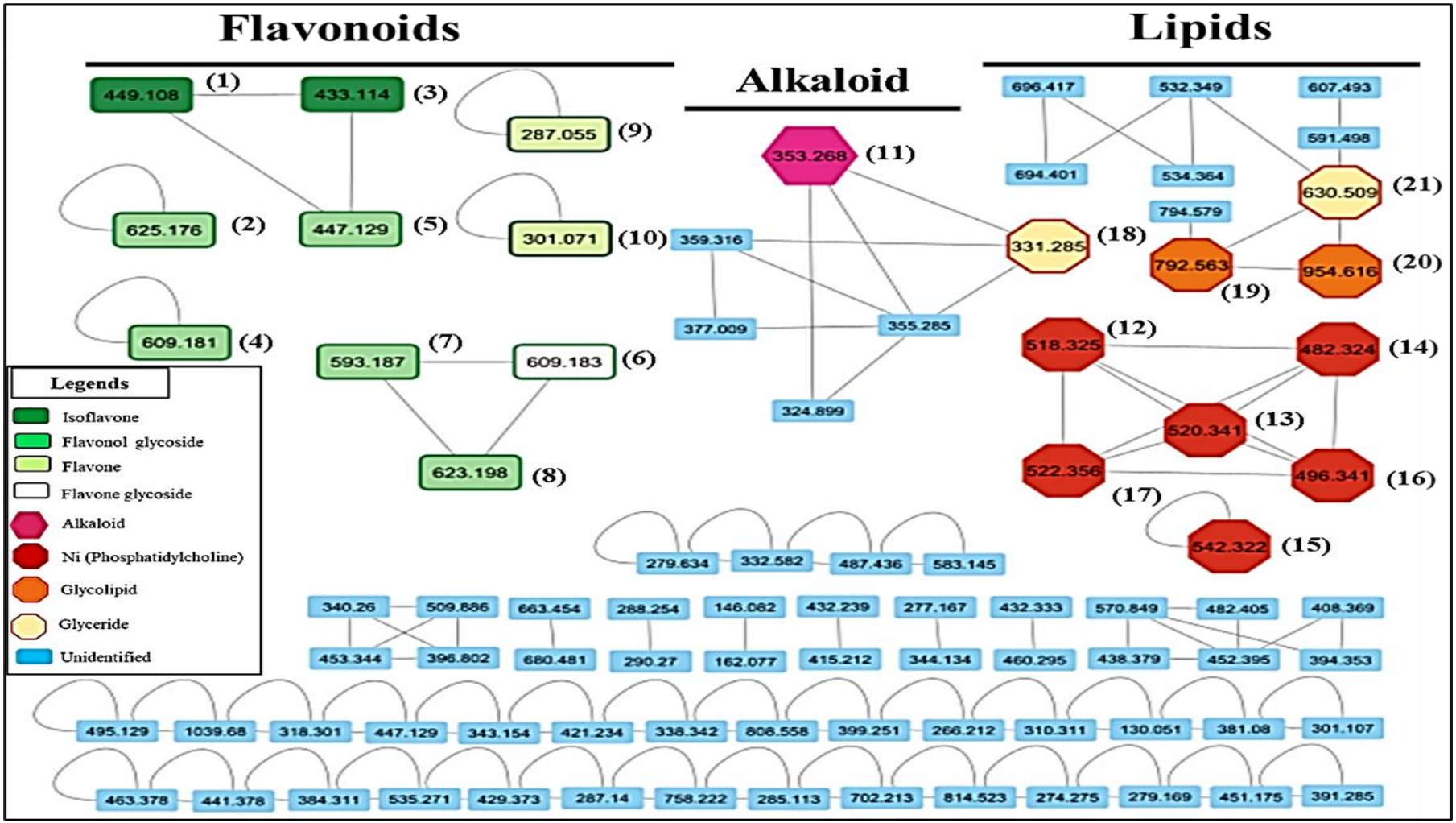


Figure 1. The HPLC-MS-MS molecular network of stem crude extract of *Senegalia polyphylla*. The annotation of different clusters represents flavonoids, alkaloid and lipids derivatives. Nodes related to the spectral library match are represented with color and shapes. Numbers outside (right side) of each node (in parentheses) correspond to table 1, while number inside the nodes represent molar masses (*m/z*).

Table 1. The chemical profile of *Senegalia polyphylla* stem crude extract analyzed by HPLC-MS-MS and partition phases (BuOH, EtOAc and EtOH) analyzed through HPLC.

No.	Compound names	Library class	RT (Min.)	*M/Z* (Spec.)	*M/Z* (Lib.)	*M/Z* (Diff.)	Sub-class	Major-class	Partition phases		
									BuOH	EtOAc	EtOH
1	3'-methoxypuerarin	Gold	11.62	449.1080	447.13	1.98	Isoflavone	Flavonoids	+	+	+
2	herbacetin-3,8-diglucopyranoside	Gold	12.61	625.1761	627.16	1.98	Flavonol glycoside	Flavonoids	+	+	+
3	3'-hydroxypuerarin	Gold	14.68	433.1130	433.11	0	Isoflavone	Flavonoids	+	+	-
4	2''-O-beta-L-galactopyranosylorientin	Gold	14.86	609.1810	611.16	1.98	Flavone glycoside	Flavonoids	+	+	+
5	luteolin-6-C-glucoside	Bronze	17.15	447.2321	449.11	1.98	Flavonol glycoside	Flavonoids	-	-	-
6	rutin	Bronze	26.04	609.1812	611.16	1.98	Flavonol glycoside	Flavonoids	+	+	-
7	kaempferol 3-O-rutinoside	Bronze	29.02	593.1844	595.16	1.98	Flavonol glycoside	Flavonoids	-	+	
8	pectolinarin	Gold	29.17	623.1981	623.2	0	Flavonol glycoside	Flavonoids	-	-	-
9	3',4',5,7-tetrahydroxyflavone	Bronze	29.31	287.0556	287.06	0	Flavone	Flavonoids	+	+	
10	6,4'-dimethoxy-3-hydroxyflavone	Bronze	31.36	301.0705	299.09	1.98	Flavone	Flavonoids	-	-	-
11	senecionine	Bronze	36.2	353.2693	352.18	1.09	Pyrrolizidine alkaloids	Alkaloid	-	-	-
12	Ni	Gold	36.76	518.3243	518.33	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-
13	Ni	Gold	38.05	520.3396	520.34	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-
14	Ni	Gold	38.27	482.3255	482.32	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-
15	Ni	Gold	38.55	542.3191	542.33	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-
16	Ni	Gold	39.23	496.3410	496.34	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-

17	Ni	Gold	40.76	522.3546	522.36	0	Phospholipid (Phosphatidylcholine)	Lipid	-	-	-
18	1-hexadecanoyl-sn-glycerol	Bronze	42.63	331.2757	331.28	0	Glyceride	Lipid	-	-	-
19	monogalactosyldiacylglycerol	Gold	46.74	792.5685	792.56	0	Glycolipid	Lipid	-	-	-
20	digalactosyldiacylglycerol	Gold	46.91	954.6244	954.62	0	Glycolipid	Lipid	-	-	-
21	diacylglycerol	Gold	49.22	630.5183	630.51	0	Glyceride	Lipid	-	-	-

Here, RT = retention time (minutes), *M/Z* (Spec.) = mass to charge ratio of the sample, *M/Z* (Lib.) = mass to charge ratio of the library (NYST), *M/Z* (Diff.) = difference in the mass to charge ratio between sample and library. Ni = not identified, BuOH = butanol partition phase, EtOAc = ethyl acetate partition phase, EtOH = hydroalcoholic residue, + = presence, - = not detected.

Table 2. GC-MS suggested phytochemicals in the stem apolar partition phases (DCM and hexane).

No	Compounds	RT (min.)	Area (%)	Chemical formulas	Classification
1	n-hexadecanoic acid	25.48	3.31	$C_{16}H_{32}O_2$	Fatty acid
2	squalene	39.61	4.43	$C_{30}H_{50}$	Terpene
3	chondrillasterol	45.95	26.94	$C_{29}H_{28}O$	Sterol
4	lup-20(29)-3-en-3one	46.69	10.67	$C_{30}H_{48}O$	Triterpene
5	lupeol	47	26.48	$C_{30}H_{50}O$	Triterpene

Here, RT = retention time (minutes)

1.3.2 Coleoptile bioassay

Senegalia polyphylla stem crude extract and their partition phases showed significant inhibitory effect on coleoptile elongation of wheat (Figure 2). Compared to negative control, the inhibition was significant at all concentrations, except 0.2 mg.mL^{-1} EtOAc partition phase. Furthermore, the inhibition was dose dependent, with maximum inhibition observed at 0.8 mg.mL^{-1}. Among all tested samples, maximum inhibition was caused by 0.8 mg.mL^{-1} Hx (41.35%), followed by DCM, BuOH, EtOH, CE and EtOAc with 36.84, 34.84, 30.9 and 27.29 and 22.04%, respectively, at 0.8 mg.mL^{-1}.

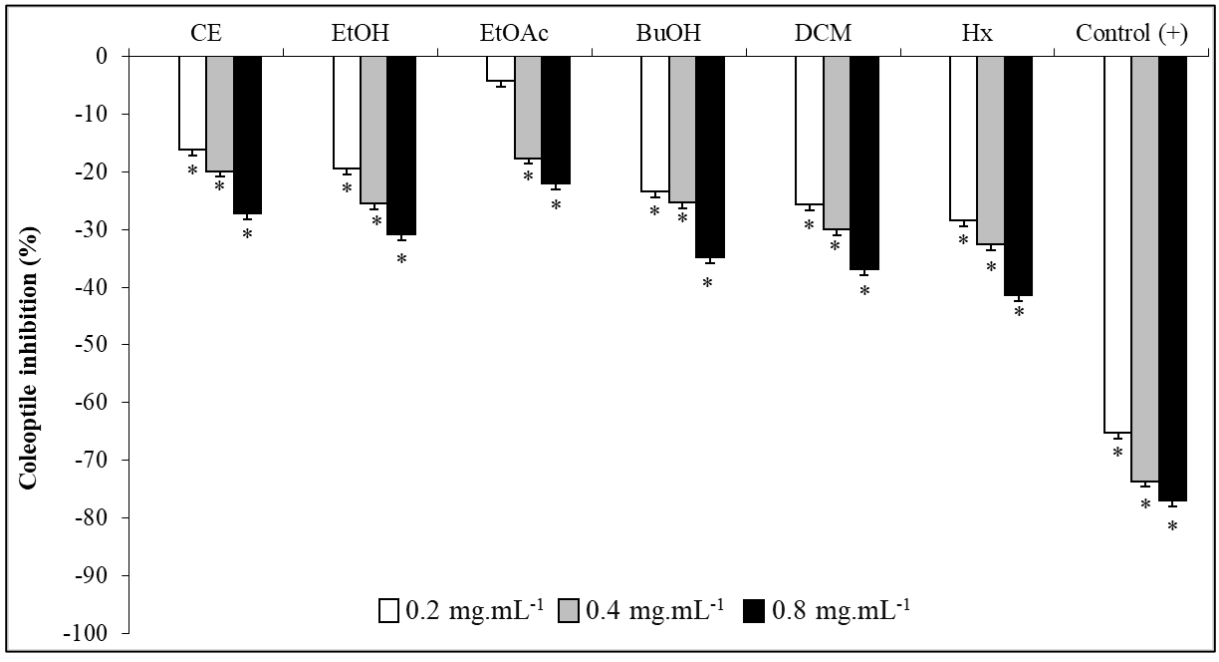


Figure 2. Inhibition percentage of coleoptile elongation of etiolated wheat. Different treatments, including stem crude extract (CE) and partition phases (Hx = hexane, DCM = dichloromethane, EtOAc = ethyl acetate, BuOH = butanol, EtOH = hydroalcoholic

residue) of *Senegalia polyphylla* and herbicide glyphosate (Control +) were implied in this bioassay. Data are mean ± SE of n=3 biological replicates. Asterisks indicate significant statistical differences from negative control at $p \leq 0.05$.

1.3.3 Germination and seedling initial growth bioassay

Since GC-MS analysis revealed a very similar composition of Hx and DCM partition phases, and the inhibition percentage at the coleoptile bioassay was also very similar, these two partition phases were pooled together to all other assays. Besides, due to the weaker effect of EtOAc partition phase in the coleoptile assay, this sample was not considered for the onward bioassays.

Crude extract (CE) and all tested partition phases of *S. polyphylla* were found to have an impact on the germination of the target species (Figure 3). As compared to the control group, CE and EtOH partition did not affect significantly the germination of lettuce and tomatoes, even at higher extract concentrations. However, the opposite was observed for BuOH and Hx/DCM. The maximum inhibitory effect was caused by Hx/DCM, with reductions of 45.83% and 55.78% observed for lettuce and tomatoes, respectively, at the concentration of 0.8 mg.mL^{-1}. Similarly, BuOH partition also affected the germination of lettuce and tomatoes, with reductions of 32.46% and 46.52%, respectively, the higher dose of 0.8 mg.mL^{-1}.

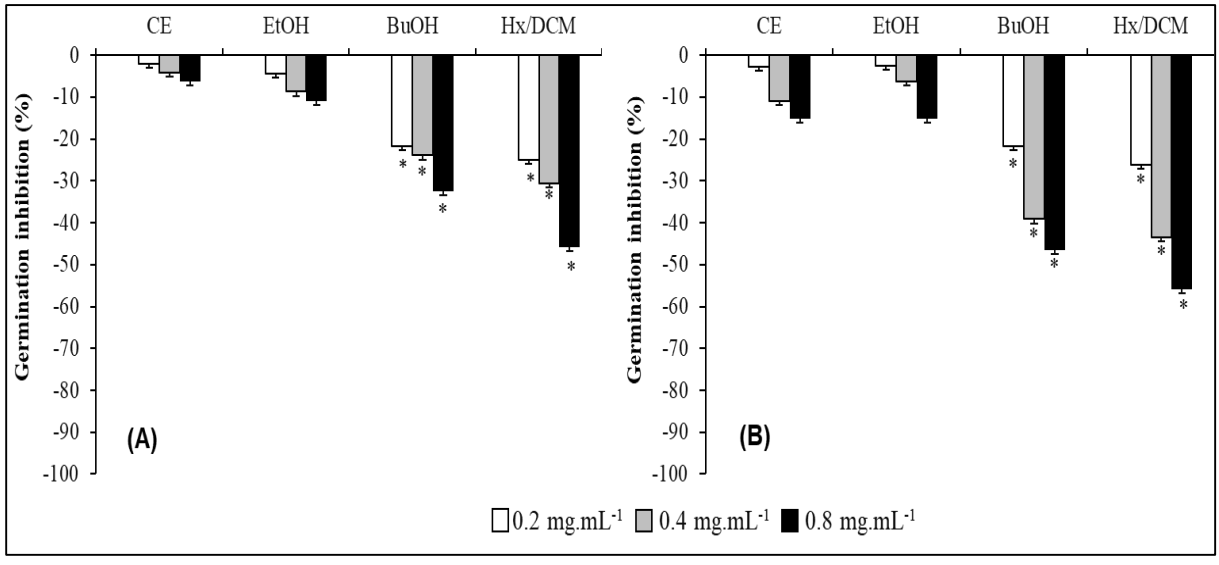


Figure 3. Effect of stem crude extract (CE) and partition phases (Hx/DCM = hexane + dichloromethane, BuOH = butanol, EtOH = hydroalcoholic residue) of *Senegalia polyphylla* on the germination of lettuce (A) and tomato (B). Data are the mean ± SE

of 5 biological replicates. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

The results revealed that BuOH and Hx/DCM partition phases significantly reduced the root and shoot length of target species in all concentrations, while CE and EtOH did not present considerable effect (Figure 4). The phytotoxic inhibition of target seedlings' root and shoot length was dose dependent. Maximum inhibition (33.1% and 47.89%) in the root length of lettuce was caused by BuOH and Hx/DCM partition phases, respectively, at higher concentration of 0.8 $mg.mL^{-1}$. On the same way, shoot length was also affected with maximum inhibition, i.e. 30.62% and 41.343%, under BuOH and Hx/DCM partition phases, respectively, at higher dose treatment (0.8 $mg.mL^{-1}$). The same pattern was found for tomato. Hx/DCM and BuOH also affect the root and shoot length of tomato seedlings at all concentrations, even harder than for lettuce. Maximum inhibition (77.34% and 48.99%) in the root length was caused by Hx/DCM and BuOH, respectively at higher extract concentration 0.8 $mg.mL^{-1}$, as well as shoot length was also inhibited (72.29% and 59.72%) by Hx/DCM and BuOH partition phases, respectively.

The stem extract of *Senegalia polyphylla* considerably affected the fresh weight of target species (Figure 4 C and F). As compared to control, CE and EtOH partition phase do not have significant effect on fresh weight of tomato and lettuce seedlings. BuOH and Hx/DCM partition phases reduced fresh weight only at higher extracts concentrations, i.e. 0.4 and 0.8 $mg.mL^{-1}$, for both target plants, while, no statistical differences were found at low concentration (0.2 $mg.mL^{-1}$). For tomato, maximum percentage reduction in the fresh weight of 62% and 46.70% was caused by Hx/DCM and BuOH partitions at 0.8 $mg.mL^{-1}$, respectively. Lettuce seedlings were less affected, with a reduction of 49.79% under Hx/DCM and 38.51% with BuOH, at 0.8 $mg.mL^{-1}$.

Based on the seedling initial growth which showed higher effect under Hx/DCM and BuOH partition phases, biochemical and anatomical analyzes were developed only with samples of these two treatments.

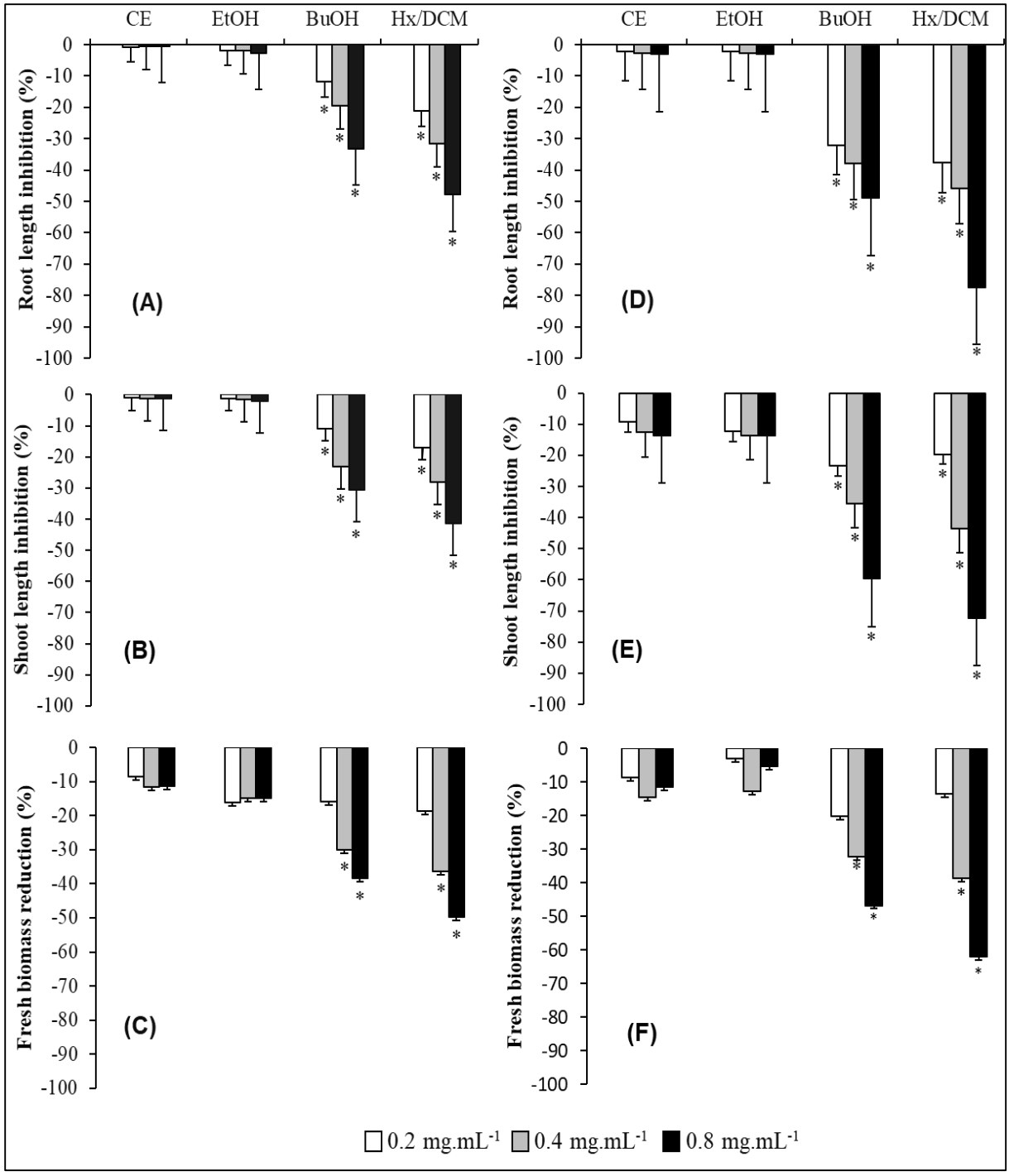


Figure 4. Root and shoot length inhibition and fresh biomass reductionof lettuce (A, B and C and tomato (D and E and F) seedlings developed under stem crude extract (CE) and partition phases (Hx/DCM = hexane + dichloromethane, BuOH = butanol, EtOH = hydroalcoholic residue) of *Senegalia polyphylla*. Data are the mean ± SE of biological replicates. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

1.3.4 Photosynthetic pigment contents

The partition phases of *Senegalia polyphylla* stem extract, namely Hx/DCM and BuOH, had a significant impact on the photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) of the target species (Figure 5). In general, tomato showed greater sensitivity to the partition phases than lettuce. On the same way, Hx/DCM presented stronger effect than BuOH.

Hx/DCM had a significant impact on the chlorophyll *a*, *b*, and carotenoid contents of lettuce seedlings in all three concentrations, with a reduction of 49.62%, 35.94% and 30.14%, respectively, when exposed to the concentration of 0.8 $mg.mL^{-1}$ (Figure 5 A). On the other hand, BuOH did not affect lettuce seedlings photosynthetic pigments at the lowest concentration (0.2 $mg.mL^{-1}$). The reduction of chlorophyll *a* was higher (39.01%) at 0.8 $mg.mL^{-1}$ than at 0.4 $mg.mL^{-1}$ (29.71%), whereas chlorophyll *b* and total carotenoids showed a similar decrease (~29.87%) at both concentrations (Figure 5 B). Similarly, the exposure of tomato seedlings to Hx/DCM resulted in a significant reduction in chlorophyll *a*, *b*, and carotenoids contents in all concentrations. The maximum reduction was observed for the concentration of 0.8 $mg.mL^{-1}$ by 61.14%, 35.96% and 42.26%, respectively (Figure 5 C). The BuOH partition phase affected all three pigments in all concentrations (Figure 5 D).

1.3.5 Antioxidant enzymes

The Hx/DCM and BuOH partition phases of stem extract of *S. polyphylla* exhibited differential effects on the antioxidant enzymes activities in the target species (Table 3). As compared to control, superoxide dismutase (SOD) activity in lettuce seedling exposed to Hx/DCM was significantly stimulated at all concentrations, but under BuOH treatment the SOD activity remain unaffected at 0.2 $mg.mL^{-1,}$ while, at higher concentrations significant upregulation was noticed. Additionally, the SOD activity was significantly upregulated in tomato seedlings under Hx/DCM and BuOH treatments at all concentrations. Moreover, compared to control, the activity of catalase (CAT) in lettuce seedlings was higher in Hx/DCM treatment at all concentrations, but was significantly upregulated only at 0.4 $mg.mL^{-1}$ BuOH treated seedlings.. For tomato, CAT activity was significantly upregulated in all concentrations of Hx/DCM treated seedlings, but no statistical difference in the CAT activity was observed in BuOH treated tomato seedlings. Curiously, while APX activity was increased in BuOH

treated lettuce seedlings, for tomato, this response was observed for Hx/DCM treated seedlings.

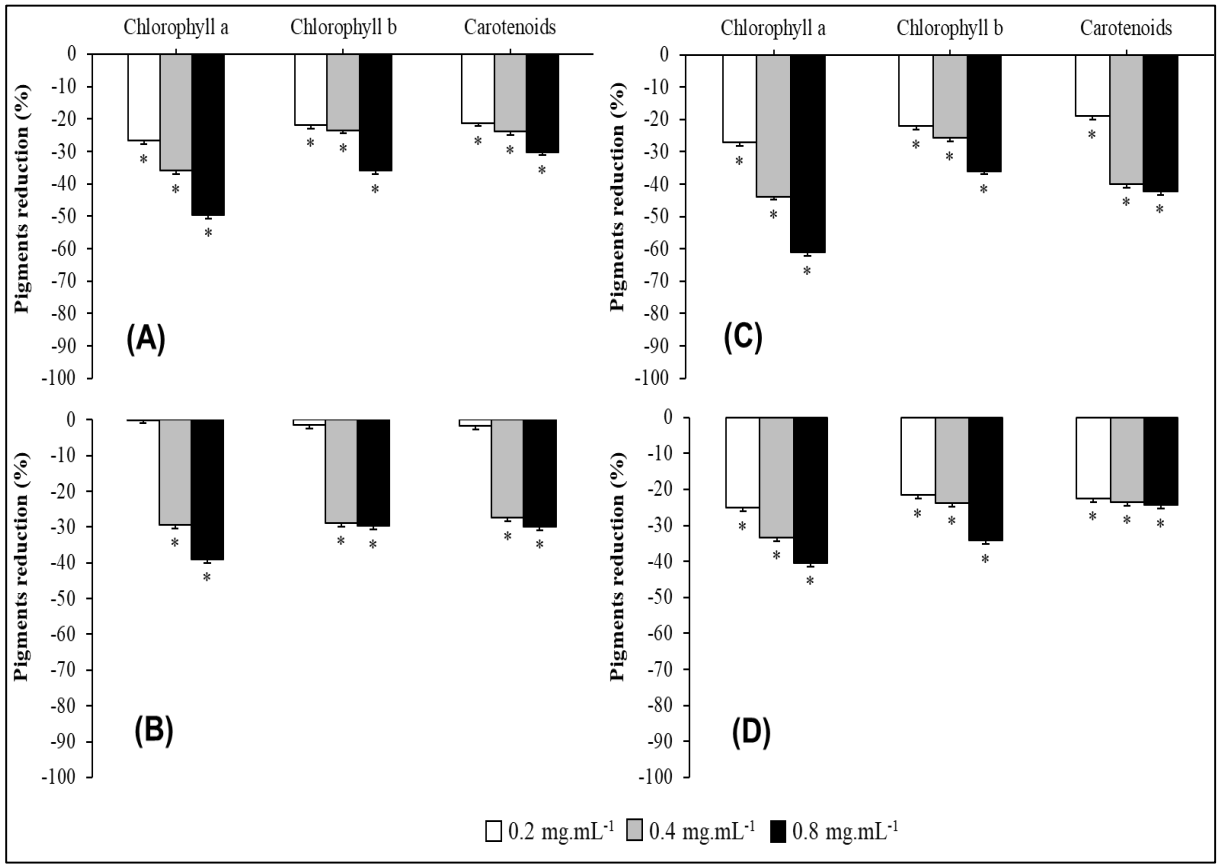


Figure 5. Effect of Hx/DCM (= hexane/dichloromethane) and BuOH (= butanol) partition phases of stem extract of *Senegalia polyphylla* on the photosynthetic pigments. A and B represent lettuce seedlings, while C and D showed tomato seedlings exposed to Hx/DCM (A and C) and BuOH (B and D). Data are means ± SE of three biological replicates. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

Table 3. Antioxidant enzymes (SOD, CAT and APX) activities in the lettuce and tomato seedlings exposed to Hx/DCM and BuOH partition phases of stem extract of *Senegalia polyphylla*).

Target plants	**Treatments**		**SOD** (U.mg^{-1} of protein)	**CAT** (µmol H_2O_2. Min^{-1}. Mg^{-1} of protein)	**APX** (µmol H_2O_2. Min^{-1}. Mg^{-1} of protein)
Lettuce	Hx/DCM	Control	1170 ± 2.42	91.73 ± 3.15	2.07 ± 1.52
		0.2 mg.mL^{-1}	1227 ± 3.11*	544.20 ± 2.14*	1.02 ± 0.06
		0.4 mg.mL^{-1}	1691.90 ± 0.41*	269.80 ± 1.19*	2.16 ± 0.28
		0.8 mg.mL^{-1}	1452 ± 1.32*	169.10 ± 5.24*	0.97 ± 0.31
	BuOH	Control	351.90 ± 2.36	279 ± 2.54	1.18 ± 2.81
		0.2 mg.mL^{-1}	273.70 ± 7.24	288.40 ± 2.10	8.07 ± 0.81*
		0.4 mg.mL^{-1}	705.70 ± 2.52*	669.90 ± 3.72*	8.05 ± 0.31*
		0.8 mg.mL^{-1}	918 ± 4.14*	262.70 ± 0.97	12.59 ± 0.71*
Tomato	Hx/DCM	Control	1555 ± 3.14	317.70 ± 3.21	2.99 ± 3.18
		0.2 mg.mL^{-1}	1984 ± 3.21*	445.80 ± 0.83*	4.36 ± 0.21*
		0.4 mg.mL^{-1}	1912 ± 2.63*	371.90 ± 1.51*	5.77 ± 0.95*
		0.8 mg.mL^{-1}	2571 ± 5.24*	393.70 ± 0.99*	4.74 ± 0.41*
	BuOH	Control	1521 ± 1.40	489.40 ± 3.36	4.70 ± 2.59
		0.2 mg.mL^{-1}	1744 ± 3.24*	398.30 ± 0.13	4.72 ± 0.4
		0.4 mg.mL^{-1}	2521 ± 4.52*	274.70 ± 2.42	1.79 ± 0.002
		0.8 mg.mL^{-1}	3074 ± 1.52*	219.30 ± 0.32	2.92 ± 1.10

BuOH = butanol partition phase, Hx/DCM = hexane/dichloromethane partition phase, SOD = superoxide dismutase, CAT = catalase, APX = ascorbic acid peroxidase. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

1.3.6 Anatomical analysis

Roots of the target seedlings from phytotoxic assay with Hx/DCM and BuOH partition phases (0.8 mg.mL^{-1}) were analyzed by light microscopy (Figures 6 and 7). Both species, lettuce and tomato, presented anatomical changes in response to treatments.

Although no visible effects on the epidermal cells was detected, the cross-section of lettuce seedlings root revealed enlarged aerenchyma tissue (AE) in both BuOH and Hx/DCM partition phases treatments (Figure 6 a, e, i). The longitudinal section of the root elongation zone showed usual arrangement of cells in control seedlings (Figure 6 b-d). Intercellular spaces (black arrows) were evident in cortical tissue and vascular cylinder of treated seedlings (Figure 6 e, g, k, l). Narrower cortical cells (*) were detected closer to aerenchyma tissue (Figure 6 f, g, j, k), maybe due the new tissue development, while wider cortical cells (#), i.e. radially elongated, appeared near to the vascular cylinder (Figure 6 f, h) mainly in BuOH treated seedlings.

Tomato seedlings exposed to Hx/DCM and BuOH partition phases also showed clear differences in the anatomical traits. As well as for lettuce, no apparent effect was observed on the epidermal cells (Figure 7 a, d, h). However, an increase in diameter was observed in the root cross-section of tomato seedlings treated with Hx/DCM, probably due to the general increase in the cortical cells size (Figure 7 h). Aerenchyma tissues (AE), as well as intercellular spaces (black arrows) appeared in the cortical tissue and in the middle of the vascular cylinder (Figure 7 d, f, h - j) from seedlings obtained in the two treatments. Distinct rounded cortical cells (*) were detected in root longitudinal section of BuOH treated seedlings (Figure 7 e, f). Furthermore, a drastic change was observed in root organization. Tomato seedlings grown with the BuOH partition phase showed early branching in relation to the root axis, with no clear distinction between elongation and differentiation zones (Figure 7 g). Aerenchyma tissues are already presented in the very young lateral roots.

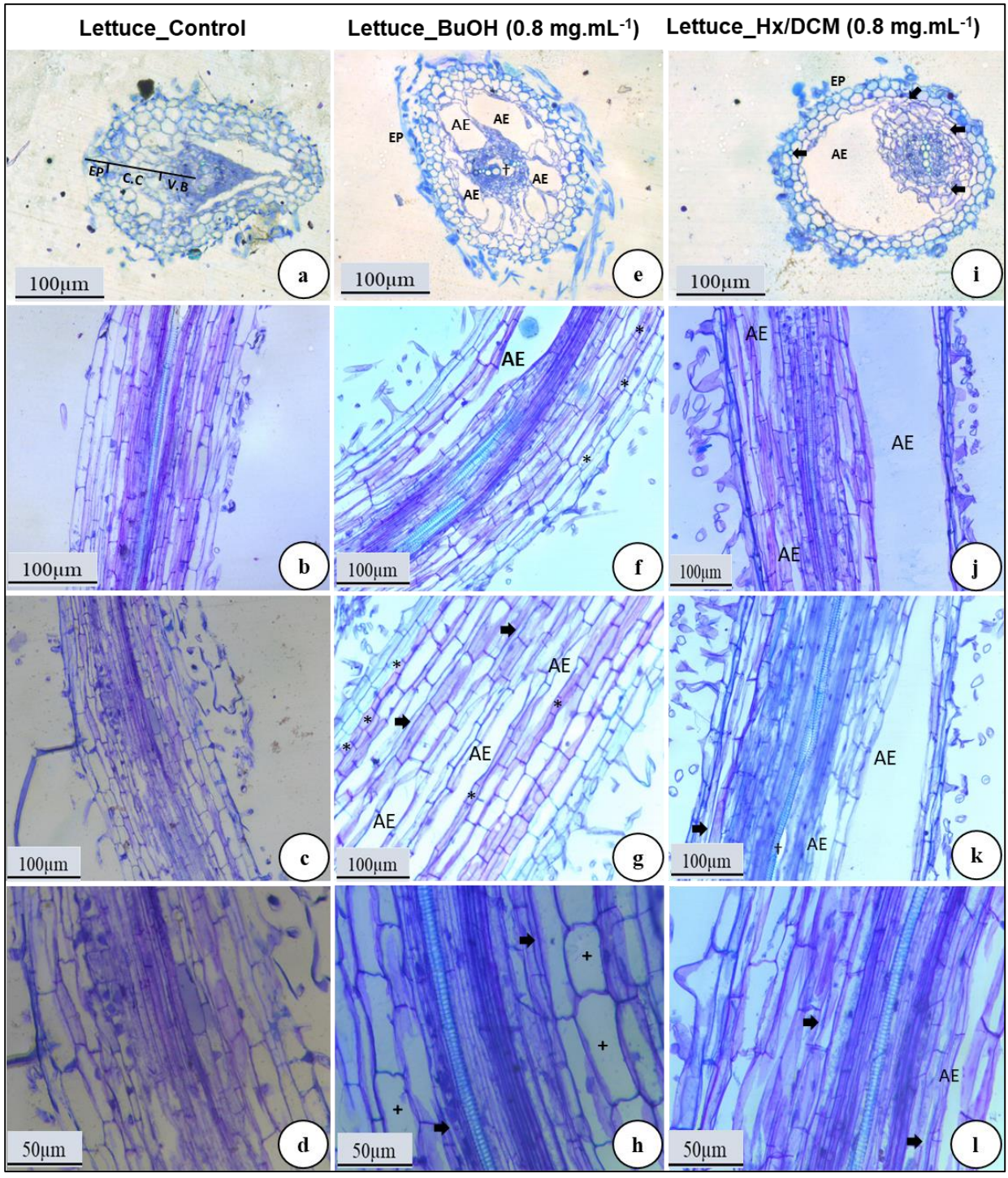


Figure 6. Anatomical characteristics of lettuce seedlings treated with butanol (BuOH) and hexane/dichloromethane (Hx/DCM) partition phases. The root cross-sections comprised the control sample (a), as well as the samples treated with BuOH (e) and Hx/DCM (i). Longitudinal view of the root was analyzed for the control sample (b-d), BuOH (f-h) and Hx/DCM treated samples (i-l). Ep = epidermis, AE = aerenchyma cells, C.C = cortical cells, V.B = vascular bundles. Here; * = smaller cells, $^{+}$ = wider cortical cells, † = intercellular spaces between vascular tissues and arrows were utilized to illustrate the presence of intercellular spaces in other tissues.

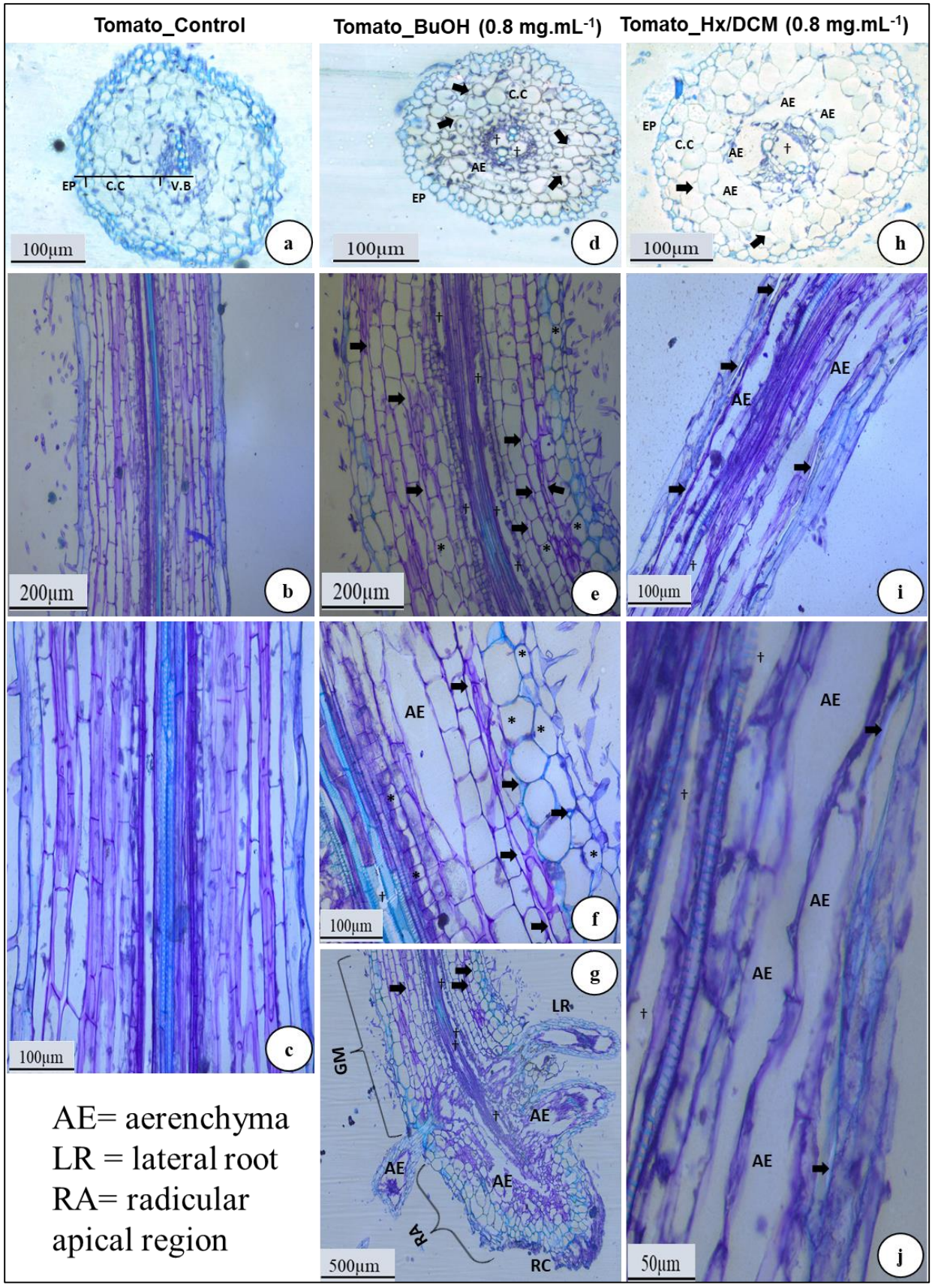


Figure 7. Anatomical characteristics of tomato seedlings treated with butanol (BuOH) and hexane/dichloromethane (Hx/DCM) partition phases. The root cross-sections comprised the

control sample (a), as well as the samples treated with BuOH (e) and Hx/DCM (i). Longitudinal view of the root was analyzed for the control sample (b-c), BuOH (e-g) and Hx/DCM treated samples (i-j). Ep = epidermis, AE = aerenchyma cells, C.C = cortical cells, V.B = vascular bundles. Here; * = rounded/smaller cells, † = intercellular spaces between vascular tissues and arrows were utilized to illustrate the presence of intercellular spaces in other tissues.

1.4 Discussion

Allelochemicals quantity and quality vary from species to species in plant clades (Soltys *et al*., 2013). However, the phytochemical profile of Fabaceae is diverse and enriched with different classes of polar and non-polar compounds (Stef *et al*., 2013). Seigler (2003) reviewed the phytochemical profile of *Acacia sensu lato* that encompasses a rich array of chemical classes, like amines, alkaloids, cyanogenic glycosides, cyclitols, fatty acids, saponins, tannins and diverse flavonoids. In the current study, stem crude extract and partition phases of *Senegalia polyphylla* were analyzed by different analytical approaches like GC-MS, HPLC-DAD and HPLC-MS/MS. Additionally, the LC-MS/MS data was further analyzed through GNPS database enabling annotate various compounds, such as flavonoid derivatives, one alkaloid and different lipids derivatives. On the same way, GC-MS analysis revealed different compounds belonging to fatty acid, sterol, terpene derivatives. Our results are in line with previous investigation of Cesarino *et al*. (2020) who also described flavonoids, triterpenes, steroids and alkane derivatives in the leaves extract of *Acacia polyphylla* (= *Senegalia polyphylla*).

Allelopathy refers to the biological phenomenon where certain plants release chemicals known as allelochemicals into the environment, influencing germination, growth and development of target species (Trezzi *et al*., 2016). To date, this is the first study highlighting the phytotoxic effect of *S. polyphylla* stem crude extract and its partition phases on wheat coleoptile bioassays CE and partition phases (except low concentration of EtOAc) exhibited significant inhibitory effect on wheat coleoptile elongation, at all concentrations. Our findings are in line with Cutler *et al*. (2000), who pointed out that coleoptile bioassay is sensitive to wide range of treatments like, extracts and isolated compounds, plant derived growth regulators (Culter, 1984) and wide range of synthetic substances (Macías *et al*., 2010). Coleoptile bioassay is very helpful for selection of more active samples to pursue fitotoxic/allelopathic assays (Chinchilla *et al*., 2017).

Conserning the germination and initial growth bioassays, Hx/DCM and BuOH partition phases significantly affect the germination rate, root and shoot length and fresh weight of lettuce and tomato seedlings. Our results are consistent with Jelassi *et al*. (2016), who investigated the chemical profile and allelopathic potential of three *Acacia* members, i.e. *Acacia cyanophylla*, *Acacia cyclops* and *Acacia mollissima*, and found that butanolic fractions inhibited significantly the growth and germination of lettuce. Besides, the flavonoids detected in BuOH partition phase have also been previously reported for their potential allelopathic effects (Macías *et al*., 1996; Spring *et al*., 1992; Qiao *et al*., 2021; Johnson *et al*., 2000). Interestingly, the compounds detected in the Hx/DCM including, n-hexadecanoic acid, squalene, chondrillasterol, lup-20(29)-3-en-3one and lupeol have also being reported for their phytotoxic effect by several researchers (Shen *et al*., 2022; Peguero *et al*., 2012; Ohsawa & Nakatani, 2005; Aguilera *et al*., 2015).

Despite the promising results observed in the coleoptile bioassay, however, CE and the EtOH partition phase did not exhibit significant effects on initial growth traits of target species. The reason for this marked effect may be attributed to the great distinction between the systems. While coleoptile is a less complex tissue, comprised of less differentiated cells, seed germination and embryo development involve more intricate systems (Einhellig, 1995) depending, for example, on seed coat thickness and permeability (Hanley & Whiting 2005). The relative simplicity of coleoptile makes it highly sensitive to a wide range of extracts/compounds (Cutler *et al*., 2000). This species-specific response to extracts/allelochemicals can shape plant composition in natural ecosystems and convey an interesting idea to design/development of selective herbicides for agriculture system (Imatomi *et al*., 2013).

The stem partition phases BuOH and Hx/DCM of *Senegalia polyphylla* induced marked anatomical changes in lettuce and tomato seedlings developed at the high treatment level (0.8 $mg.mL^{-1}$). These changes included the appearance of aerenchyma tissues, wider cortical cells and the formation of empty spaces between the cells and inside the vascular bundles. Aerenchyma formation in response to extreme environmental conditions has already been described as an important adaptation to stressed conditions for plants to provide maximum flow of gases (O_2, N_2 and CO_2) with in/out of root system (Evans, 2004; Wegner, 2010; Takahashi *et al*., 2014). In the current study, the appearance of aerenchyma tissue, intercellular spaces and smaller

cortex cells may be related to fresh biomass reduction. Celedonio *et al*. (2017) previously stated that aerenchyma tissues production leads to root and shoot biomass reductions in the wheat and barley cultivars under waterlogging system. Aerenchyma tissue formation were observed even in the new lateral roots in tomato seedlings. Retig *et al*. (1972) also observed significant inhibition in the cell elongation, disruption of epidermis and changes in arrangement of tissues in the root tissue of cabbage and tomato seedlings under effect of *Datura stramonium* L., *Setria viridis* L, *Abutilon theophrasti* Medic. and *Brassica kaber* DC. Mayworm *et al*. (2017) studing the effects of essential oils from Brazilian propolis on lettuce radicle cells elongation show that the extract acted preventing root to protruding, and intercellular spaces appeared between the cell layers.

Chlorophylls and carotenoids are synthesized within chloroplasts and are essential pigments for photosynthesis of plants (Yue *et al*., 2021). Therefore, the quantification of these photosynthetic pigments in plants is widely used as a reliable marker for assessing the physiological state and evaluating the impact of environmental stresses (Queiroz *et al*., 2017; Neves *et al*., 2009). In our study, photosynthetic pigments including chlorophyll *a*, *b* and carotenoids contents decreased in tomato and lettuce seedlings under Hx/DCM and BuOH partition phases. Our findings agree with previous results of Batish *et al*. (2007) who described a decrease in chlorophyll amounts, associated with growth reduction, in two legume species under *Chenopodium murale* L. phytotoxic effect. Photosynthetic pigments are essential for development of plants, however, reduction in these pigments can be related to initial growth inhibition observed for both tomato and lettuce target plants and also for decrease in fresh biomass (Einhellig, 1995; El-Sheekh *et al*., 2010).

Plants subjected to biotic and abiotic stresses produces an excess of reactive oxygen species (ROS) that surpasses their antioxidant defense potential (Gniazdowska *et al*., 2015). However, plants possess a cellular enzymatic system that regulates the levels of ROS and high level of hydrogen peroxide (H_2O_2). These enzymes comprise of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), thioredoxin (Trx) among others (Mansoor *et al*., 2022).

In the present study, SOD activity in the tomato and lettuce seedlings exposed to BuOH and Hx/DCM partition phases were up regulated at all concentrations, following the same pattern observed in other studies (Haithloul *et al*., 2022; Tewari *et*

al., 2021; Lara-Nuñez *et al*., 2006; Oracz *et al*., 2007). Probably, the allelochemicals present at the partition phases cause imbalance homeostasis, leading to an increase in this antioxidant enzyme in the target plants (Lara-Nuñez *et al.*, 2006). On the other hand, CAT and APX did not exhibit homogeneous patterns. Seedlings of both target species, lettuce and tomato, grown under Hx/DCM, presented higher CAT activity, while APX activity increased in BuOH treated lettuce seedlings and in Hx/DCM tomato seedlings. Haithloul *et al*. (2022) noticed increased CAT and APX activities in crops (*Raphanus sativus* L., *Triticum aestivum* L., *Eruca sativa* Miller. and *Hordeum vulgare* L.) submitted to low concentrations of *Acacia salinga* extract. However, these activities decreased in plants grown under high extract concentration. This decrease was not observed in the present study.

1.5 Conclusion

The results of this study demonstrated the significant inhibitory effects of *Senegalia polyphylla* stem crude extract and its partition phases on wheat coleoptile growth bioassay, except for the EtOAc partition phase at lower concentrations. However, no significant effects were observed for the CE and EtOH partition phase on the growth and initial development of lettuce and tomato seedlings. In contrast, the Hx/DCM and BuOH partition phases exhibited significant inhibitory effects on the germination rate, root and shoot length, as well as in reduction of the fresh biomass of tomato and lettuce seedlings. Furthermore, these two partition phases caused significant reduction of chlorophyll *a*, *b*, and carotenoid contents, indicating their negative impact on photosynthetic pigments. On the same way, these two treatments also affected antioxidant enzymes activities and anatomical traits of the target species. The chemical profiling of the *S. polyphylla* stem crude extract revealed a diverse range of phytochemicals, including lipids, sterol, terpenes, alkaloid and phenolic derivatives like flavonoid glycosides. These findings provide valuable insights into the potential allelopathic effects of *S. polyphylla* stem extract and its partition phases, highlighting their influence on other plants. However, further research is recommended to unrevealed potential bioactive compounds and to monitore its impact on important crops, under field conditions.

1.6 References

Abdel-Farid, I.B., Sheded, M.G. and Mohamed, E.A., 2014. Metabolomic profiling and antioxidant activity of some *Acacia* species. *Saudi Journal of Biological Sciences*, *21*(5), pp.400-408.

Aguilera, N., Becerra, J., Guedes, L.M., Villasenor-Parada, C., Gonzalez, L. and Hernandez, V., 2015. Allelopathic effect of the invasive *Acacia dealbata* Link (Fabaceae) on two native plant species in south-central Chile. *Gayana Botánica*, 72(2), pp.231-239.

ALHaithloul, H.A.S., Khan, M.I., Musa, A., Ghoneim, M.M., ALrashidi, A.A., Khan, I., Azab, E., Gobouri, A.A., Sofy, M.R., El-Sherbiny, M. and Soliman, M.H., 2022. Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. *PeerJ*, *10*, p.e13623.

Al-Wakeel, S.A.M., Gabr, M.A., Hamid, A.A. and Abu-El-Soud, W.M., 2007. Allelopathic effects of *Acacia nilotica* leaf residue on *Pisum sativum* L. *Allelopathy Journal*, 19(2), p.411.

Barros, M.J.F. and Morim, M.P., 2014. *Senegalia* (Leguminosae, Mimosoideae) from the Atlantic Domain, Brazil. *Systematic Botany*, *39*(2), pp.452-477.

Batish, D.R., Lavanya, K., Singh, H.P. and Kohli, R.K., 2007. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regulation*, 51, pp.119-128.

Blum, U. and Blum, U., 2011. *Plant–plant allelopathic interactions* (pp. 1-7). *Springer Netherlands*.

Cesarino, E.C., Gomes, J.O.D.S., Lessa, E.V., Carvalho Junior, A.R.D., Vieira, I.J., Carvalho, M.G.D., Silva, T. and Ferreira, R.O., 2020. Chemical composition and

antioxidant activity of *Acacia Polyphylla* (Fabaceae). *Revista Virtual de Quimica*, 12(2), pp.424-432.

Chinchilla, N., Guerrero-Vásquez, G.A., Varela, R.M., Molinillo, J.M. and Macías, F.A., 2017. Phytotoxic studies of naphthoquinone intermediates from the synthesis of the natural product Naphthotectone. *Research on Chemical Intermediates*, *43*, pp.4387-4400.

Chou, C.H., Fu, C.Y., Li, S.Y. and Wang, Y.F., 1998. Allelopathic potential of *Acacia confusa* and related species in Taiwan. *Journal of Chemical Ecology*, *24*, pp.2131-2150.

Cutler, H.G., 1984. A fresh look at the wheat coleoptile bioassay. In *Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America* (pp. 1-9).

Cutler, S.J., Hoagland, R.E. and Cutler, H.G., 2000. Evaluation of selected pharmaceuticals as potential herbicides: bridging the gap between agrochemicals and pharmaceuticals. In *Allelopathy in Ecological Agriculture and Forestry: Proceedings of the III International Congress on Allelopathy in Ecological Agriculture and Forestry, Dharwad, India, 18–21 August 1998* (pp. 129-137). Springer Netherlands.

de San Celedonio, R.P., Abeledo, L.G., Mantese, A.I. and Miralles, D.J., 2017. Differential root and shoot biomass recovery in wheat and barley with transient waterlogging during preflowering. *Plant and Soil*, *417*, pp.481-498.

Einhellig, F.A., 1995. Mechanism of action of allelochemicals in allelopathy.

El-Sheekh, M.M., Khairy, H.M. and El-Shenody, R.A., 2010. Allelopathic effects of cyanobacterium *Microcystis aeruginosa* Kützing on the growth and photosynthetic pigments of some algal species. *Allelopathy Journal*, *26*(2).

Evans, D.E., 2004. Aerenchyma formation. *New phytologist*, *161*(1), pp.35-49.

Gebrekiros, S. T. (2016). Factors affecting stream fish community composition and habitat suitability. *Journal of Aquaculture & Marine Biology*, *4*(2), 00076.

Geider, R.J., Delucia, E.H., Falkowski, P.G., Finzi, A.C., Grime, J.P., Grace, J., Kana, T.M., La Roche, J., Long, S.P., Osborne, B.A. and Platt, T., 2001. Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global change biology*, *7*(8), pp.849-882.

Gniazdowska, A., Krasuska, U., Andrzejczak, O. and Soltys, D., 2015. Allelopathic compounds as oxidative stress agents: YES or NO. *Reactive oxygen and nitrogen species signaling and communication in plants*, pp.155-176.

Han, X., Cheng, Z., Meng, H., Yang, X. and Ahmad, I., 2013. Allelopathic effect of decomposed garlic (*Allium sativum* L.) stalk on lettuce (*Lectuca sativa* var. crispa L.). *Pakistan Jouranl Botany*, *45*(1), pp.225-233.

Hanley, M.E. and Whiting, M.D., 2005. Insecticides and arable weeds: effects on germination and seedling growth. *Ecotoxicology*, *14*, pp.483-490.

Hasanuzzaman, M., Bhuyan, M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M. and Fotopoulos, V., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*(8), p.681.

Hoque, A.T.M.R., Ahmed, R., Uddin, M.B. and Hossain, M.K., 2003. Allelopathic effects of different concentration of water extract of *Eupatorium odoratum* leaf on germination and growth behavior of six agricultural crops. *OnLine Journal of Biological Science*, *3*(8), pp.741-750.

Hussain, M.I., El-Sheikh, M.A. and Reigosa, M.J., 2020. Allelopathic potential of aqueous extract from *Acacia melanoxylon* R. Br. on *Lactuca sativa*. *Plants*, *9*(9), p.1228.

Hussain, M.I., Gonzalez, L. and Reigosa, M.J., 2011. Allelopathic potential of *Acacia melanoxylon* on the germination and root growth of native species. *Weed Biology and Management, 11*(1), pp.18-28.

Imatomi, M., Novaes, P., Matos, A.P., Gualtieri, S.C., Molinillo, J.M., Lacret, R., Varela, R.M. and Macías, F.A., 2013. Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves. *Biochemical Systematics and Ecology*, *46*, pp.29-35.

Jadhav, B.B. and Gaynar, D.G., 1995. Effect of *Casuarina equisetifolia* JR leaf litter leachates on germination and seedling growth of rice and cowpea. *Allelopathy Journal*, *2*(1), pp.105-108.

Jelassi, A., El Ayeb-Zakhama, A., Nejma, A.B., Chaari, A., Harzallah-Skhiri, F. and Jannet, H.B., 2016. Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. *Industrial crops and products*, *83*, pp.339-345.

Johansen, D.A., 1940. *Plant microtechnique*. McGraw-Hill Book Company, Inc: London; 530p.

Johnson, G.A., Mantha, S.V. and Day, T.A., 2000. A spectrofluorometric survey of UV-induced blue-green fluorescence in foliage of 35 species. *Journal of Plant Physiology*, *156*(2), pp.242-252.

Kalaji, M.H. and Guo, P., 2008. Chlorophyll fluorescence: a useful tool in barley plant breeding programs. *Photochemistry research progress*, 29, pp.439-463.

Karnovsky, M. (1965). A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology*. 27, 137–138.

Keating, K. Irwin. "Allelopathy: principles, procedures, processes, and promises for biological control." *Advances in agronomy* 67 (1999): 141-231.

LARA-NUÑEZ, A.U.R.O.R.A., ROMERO-ROMERO, T.E.R.E.S.A., Ventura, J.L., Blancas, V., Anaya, A.L. and CRUZ-ORTEGA, R.O.C.I.O., 2006. Allelochemical stress causes inhibition of growth and oxidative damage in *Lycopersicon esculentum* Mill. *Plant, Cell & Environment*, *29*(11), pp.2009-2016.

Li, J., Zhao, T., Chen, L., Chen, H., Luo, D., Chen, C., Miao, Y. and Liu, D., 2022. *Artemisia argyi* allelopathy: a generalist compromises hormone balance, element absorption, and photosynthesis of receptor plants. *BMC Plant Biology*, 22(1), pp.1-17.

Machado, S.W., de Oliveira, C.F.R., Bezerra, C.D.S., Machado Freire, M.D.G., Regina Kill, M., Machado, O.L.T., Marangoni, S. and Macedo, M.L.R., 2013. Purification of a Kunitz-type inhibitor from *Acacia polyphylla* DC seeds: Characterization and insecticidal properties against *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae). *Journal of agricultural and food chemistry*, *61*(10), pp.2469-2478.

Macías, F.A., Torres, A., Molinllo, J.G., Varela, R.M. and Castellano, D., 1996. Potential allelopathic sesquiterpene lactones from sunflower leaves. *Phytochemistry*, *43*(6), pp.1205-1215.

Mansoor, S., Ali Wani, O., Lone, J.K., Manhas, S., Kour, N., Alam, P., Ahmad, A. and Ahmad, P., 2022. Reactive oxygen species in plants: from source to sink. *Antioxidants*, *11*(2), p.225.

Mayworm, M.A., dos Santos, A.B., Melo-de-Pinna, G.F., Salatino, A., Salatino, M.L. and Demarco, D., 2017. Monoterpenes from the essential oil from Brazilian propolis affect seedling cellular elongation. *Brazilian Journal of Botany*, *40*, pp.609-615.

Miller, J.T. and Seigler, D., 2012. Evolutionary and taxonomic relationships of *Acacia sl* (Leguminosae: Mimosoideae). *Australian Systematic Botany*, *25*(3), pp.217-224.

Moore, C.M., Mills, M.M., Arrigo, K.R., Berman-Frank, I., Bopp, L., Boyd, P.W., Galbraith, E.D., Geider, R.J., Guieu, C., Jaccard, S.L. and Jickells, T.D., 2013. Processes and patterns of oceanic nutrient limitation. *Nature geoscience*, *6*(9), pp.701-710.

Muzell Trezzi, M., Vidal, R.A., Balbinot Junior, A.A., von Hertwig Bittencourt, H. and da Silva Souza Filho, A.P., 2016. Allelopathy: driving mechanisms governing its activity in agriculture. *Journal of Plant Interactions*, 11(1), pp.53-60.

Neves, N.R., Oliva, M.A., da Cruz Centeno, D., Costa, A.C., Ribas, R.F. and Pereira, E.G., 2009. Photosynthesis and oxidative stress in the restinga plant species *Eugenia uniflora* L. exposed to simulated acid rain and iron ore dust deposition: potential use in environmental risk assessment. *Science of the total environment*, 407(12), pp.3740-3745.

Novaes, P., Torres, P.B. and dos Santos, D.Y.A.C., 2016. Biological activities of Annonaceae species extracts from Cerrado. *Brazilian Journal of Botany*, *39*, pp.131-137.

O'Brien, T., Feder, N. and McCully, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*, *59*, pp.368-373.

Ohsawa, R. and Nakatani, C., 2005. Inter-and intra-cultivar variations in the allelopathic effect of leaf aqueous extract of buckwheat (*Fagopyrum esculentum* Moench.) on the growth of lettuce seedling. *Fagopyrum*, 22, pp.21-24.

Oracz, K., Bailly, C., Gniazdowska, A., Côme, D., Corbineau, F. and Bogatek, R., 2007. Induction of oxidative stress by sunflower phytotoxins in germinating mustard seeds. *Journal of chemical ecology*, *33*, pp.251-264.

Peguero, G., Lanuza, O.R., Savé, R. and Espelta, J.M., 2012. Allelopathic potential of the neotropical dry-forest tree *Acacia pennatula* Benth.: inhibition of seedling

establishment exceeds facilitation under tree canopies. *Plant ecology*, 213, pp.1945-1953.

Pérez Chaca, M.V., Vigliocco, A., Reinoso, H., Molina, A., Abdala, G., Zirulnik, F. and Pedranzani, H., 2014. Effects of cadmium stress on growth, anatomy and hormone contents in *Glycine max* (L.) Merr. *Acta Physiologiae Plantarum*, *36*, pp.2815-2826.

Qiao, Z., Han, L., Liu, X., Dai, H., Liu, C., Yan, M., Li, W., Han, W., Li, X., Huang, S. and Gao, B., 2021. Extraction, radical scavenging activities, and chemical composition identification of flavonoids from sunflower (*Helianthus annuus* L.) receptacles. *Molecules*, *26*(2), p.403.

Queiroz, M.I., Fernandes, A.S., Deprá, M.C., Jacob-Lopes, E. and Zepka, L.Q., 2017. Introductory chapter: Chlorophyll molecules and their technological relevance. *Chlorophyll; Jacob-Lopes, E., Zepka, LQ, Queiroz, MI, Eds*, pp.1-5.

Reigosa, M.J. and Carballeira, A., 2016. Phenological phase influences allelopathic potential of water leachates of *Acacia dealbata* obtained using simulated rain. *Journal. Allelochemical. Interactions*, *2*, pp.9-15.

Retig, B., Holm, L.G. and Struckmeyer, B.E., 1972. Effects of weeds on the anatomy of roots of cabbage and tomato. *Weed Science*, *20*(1), pp.33-36.

Rewald, B., Razaq, M., Lixue, Y., Li, J., Khan, F. and Jie, Z., 2018. Root order-based traits of Manchurian walnut & larch and their plasticity under interspecific competition. *Scientific Reports*, *8*(1), pp.1-14.

Rial, C., Novaes, P., Varela, R.M., G. Molinillo, J.M. and Macias, F.A., 2014. Phytotoxicity of cardoon (*Cynara cardunculus*) allelochemicals on standard target species and weeds. *Journal of Agricultural and food chemistry*, *62*(28), pp.6699-6706.

Rice, E.L., 1984. Manipulated ecosystems: Roles of allelopathy in agriculture. *Allelopathy. Academic Press, New York*, pp.1-422.

Sala-Carvalho, W.R., Montessi-Amaral, F.P., Esposito, M.P., Campestrini, R., Rossi, M., Peralta, D.F., Furlan, C.M., 2022. Metabolome of *Ceratodon purpureus* (Hedw.) Brid., a cosmopolitan moss: the influence of seasonality. *Planta*, *255*: 77.

Scabora, M. H., Maltoni, K. L., & Cassiolato, A. M. R. (2011). Mycorrhizal association in tree species, microbial activity and soil fertility on. *Ciência Florestal*, *21*(2), 289-301.

Seigler, D.S., 2003. Phytochemistry of *Acacia—sensu* lato. *Biochemical systematics and ecology*, *31*(8), pp.845-873.

Shen, S., Ma, G., Xu, G., Li, D., Jin, G., Yang, S., Clements, D.R., Chen, A., Wen, L., Zhang, F. and Ye, M., 2022. Allelochemicals identified from sweet potato (*Ipomoea batatas*) and their allelopathic effects on invasive alien plants. *Frontiers in Plant Science*, 13, p.955.

Soltys, D., Krasuska, U., Bogatek, R. and Gniazdowska, A., 2013. Allelochemicals as bioherbicides—Present and perspectives. In *Herbicides-Current research and case studies in use*. IntechOpen.

Souza-Alonso, P., Puig, C.G., Pedrol, N., Freitas, H., Rodríguez-Echeverría, S. and Lorenzo, P., 2020. Exploring the use of residues from the invasive *Acacia* sp. for weed control. *Renewable Agriculture and Food Systems*, *35*(1), pp.26-37.

Spring, O., Rodon, U. and Macias, F.A., 1992. Sesquiterpenes from noncapitate glandular trichomes of *Helianthus annuus*. *Phytochemistry*, *31*(5), pp.1541-1544.

Stef, R., Bostan, C., Butu, A., Ortan, A., Rodino, S. and Butu, M., 2013. Comparative characteristics of *Lupinus perennis* L. under allelochemical sorgoleone stress. *Romanian Biotechnological Letters*, *18*(3), pp.8327-8332.

Takahashi, H., Yamauchi, T., Colmer, T.D. and Nakazono, M., 2014. Aerenchyma formation in plants. *Low-Oxygen Stress in Plants: Oxygen Sensing and Adaptive Responses to Hypoxia*, pp.247-265.

Tewari, R.K., Yadav, N., Gupta, R. and Kumar, P., 2021. Oxidative stress under macronutrient deficiency in plants. *Journal of Soil Science and Plant Nutrition*, *21*, pp.832-859.

Torres, P.B., Chow, F., Furlan, C.M., Mandelli, F., Mercadante, A. and Santos, D.Y.A.C.D., 2014. Standardization of a protocol to extract and analyze chlorophyll a and carotenoids in *Gracilaria tenuistipitata* Var. Liui. Zhang and Xia (Rhodophyta). *Brazilian Journal of Oceanography*, *62*, pp.57-63.

Wegner, L.H., 2010. Oxygen transport in waterlogged plants. *Waterlogging signalling and tolerance in plants*, pp.3-22.

Yuan, Z., Ni, X., Chen, C., Zhang, S., Chen, X., Yang, Z. and Li, C., 2022. Effects of different water conditions on the biomass, root morphology and aerenchyma formation in bermudagrass (*Cynodon dactylon* (L.) Pers). *BMC Plant Biology*, *22*(1), pp.1-14.

Yue, C., Wang, Z. and Yang, P., 2021. The effect of light on the key pigment compounds of photosensitive etiolated tea plant. *Botanical Studies*, 62(1), pp.1-15.

Zhang, S., Sun, S.W., Shi, H.L., Zhao, K., Wang, J., Liu, Y., Liu, X.H. and Wang, W., 2020. Physiological and biochemical mechanisms mediated by allelochemical isoliquiritigenin on the growth of lettuce seedlings. *Plants*, *9*(2), p.245.

Zhi-hui, C., Chun-hui, W., Xue-mei, X. and Khan, M.A., 2011. Allelopathic effects of decomposing garlic stalk on some vegetable crops. *African Journal of Biotechnology*, *10*(69), p.15514.7.

Zohaib, A., Abbas, T. and Tabassum, T., 2016. Weeds cause losses in field crops through allelopathy. *Notulae Scientia Biologicae*, *8*(1), pp.47-56.

CHAPTER 2

Phytochemical profile and phytotoxic effect of *Senegalia polyphylla* leaves crude extract and partition phases on initial growth and development of target species

Abstract

Allelopathy, an intriguing ecological phenomenon, involves complex chemical interactions among plants, resulting either favourable and/or adverse effects on target plants. The aim of this study was to investigate chemical profile of *Senegalia polyphylla* leaves and partition phases and its effect on the growth and development of target species. The crude extract (CE) and partition phases, i.e. hexane (Hx), dichloromethane (DCM), butanol (BuOH) and hydroalcoholic residues (EtOH), delayed wheat coleoptile elongation, with the maximum inhibition observed for Hx partition phase. CE and EtOH partition phase did not affect germination, root and shoot and fresh biomass of target species, while Hx/DCM exhibited significant phytotoxic effects, followed by BuOH. Furthermore, while both BuOH and Hx/DCM caused significant reduction in chlorophyll *a*, *b* and carotenoid contents of the target species, antioxidant enzymes showed differential regulation. SOD activity was upregulated at all concentration, CAT was not affected and APX showed increased activity only in tomato seedlings grown under Hx/DCM and BuOH. Phytochemical investigation revealed the presence of terpenes, fatty acids and carboxylic acid derivatives in the Hx/DCM partition phase based on GC-MS analysis. Additionally, HPLC-DAD also confirmed the presence of different flavonoids derivatives, such as isoflavone, flavonol glycoside, and flavone derivatives in BuOH, EtOAc and EtOH. The current study finds significant phytotoxic effect of *S. polyphylla* leaf extract. However, further studies are advised to isolate active compounds and monitor its effect on important crops under field conditions.

Key words; allelochemicals, coleoptile bioassay, GC-MS, *Senegalia,* antioxidant enzymes, pigments

2.1 Introduction

Allelopathy is a captivating ecological phenomenon, wherein donor plants release allelochemicals that influences germination, growth and development of target species (Cheng & Cheng, 2015). The effect caused by allelochemicals are either inhibitory and/or stimulatory, however, dependent on time of exposure, concentration, tissue types and species involved (donor and acceptor species) (Hussain *et al*., 2021; Sangeetha & Baskar, 2015). Different phytotoxic effects exhibited by allelochemicals in target species are reduction of photosynthetic pigments, seed germination, antioxidant enzymes and growth traits (e.g. fresh biomass, root and shoot length) (Zhou & Yu, 2006; Zohaib *et al*., 2016; Li *et al*., 2022). Allelochemicals are present in all plants, their quality and quantity differ among various tissues of the donor plants, including roots, seeds, leaves, bark, flowers, pods, fruits, and stems (Kato-Noguchi & Kurniadie, 2022). However, leaves emerge as the richest source, contributing significantly to the allelopathic effects observed in the target species (Skinner *et al*., 2012). These compounds are released through various processes, including the decomposition of residues, volatilization, leaching and root exudations (Hierro & Callaway, 2003). Confirming and identifying these allelochemicals present a complex and challenging task. Nevertheless, diverse analytical techniques, such as HPLC, GC-MS, HPLC-MS/MS and NMR are commonly employed for this purpose (Macías *et al*., 2019).

The genus *Acacia* is distributed worldwide (Doyle & Luckow, 2003) and assumes significant importance due to its extensive usage in treating various ailments, as well as to its phytotoxic effect on various crops species (Carballeira & Reigosa, 1999; Hussain *et al*., 2020; Chou *et al*., 1998; Al-Wakeel *et al*., 2007). Recently, Miller & Seigler (2012) segregated some species of *Acacia* into *Senegalia* based on molecular and morphological characters. Due that, most of the crucial information concerning allelopathic and phytochemical data are only available for *Acacia*.

Hoque *et al*. (2003) demonstrated inhibitory effect of *Acacia auriculiformis* A. Cunn. ex Benth. leaf aqueous extract on the growth and development of *Brassica juncea* (L.) Czern., *Vigna mungo* (L.) Hepper, *Phaseolus mungo* L., *Raphanus sativus* L., *Vigna unguiculata* L. and *Cicer arietinum* L. Additionally, Jelassi *et al*. (2016) demonstrated that a triterpenoid saponin (mollisside B), present in the butanol fraction of *Acacia decurrens* (J.C.Wendl.) Willd., affect the germination, root and shoot length

of lettuce. El-Khawas & Shehata (2005) reported the phytotoxic effects of leaf leachate from the *Acacia nilotica* (L.) Willd. ex Delile and *Eucalyptus rostrata* Schltdl. in the germination, root and shoot length and chlorophyll contents of *Zea mays* L. and *Phaseolus vulgaris* L. seedlings. Allelopathic effect leachate extracts from *Acacia saligna* (Labill.) H.L.Wendl. on crops *Raphanus sativus* L., *Triticum aestivum* L., *Eruca sativa* Miller. and *Hordeum vulgare* L. were observed through growth traits, antioxidant enzymes and chlorophyll contents (Haithloul *et al*., 2022).

Senegalia polyphylla (DC.) Britton & Rose, known as "monjoleiro," is a native tree in Brazil widely used in the restoration of degraded areas (Barbosa *et al*., 2022), woodworking (Lima *et al*., 2022) and reforestation (Meli *et al*., 2018). *S. polyphylla* resin has been traditionally used for the treatment of cough and a protein, extracted from its seeds, showed potential insecticidal activity against *Anagasta kuehniella* (Zeller) (Machado *et al*., 2013). Furthermore, Cesarino *et al*. (2020) investigated the phytochemical composition of *Acacia polyphylla* (= *Senegalia polyphylla*) describing five flavonoids (luteolin, isovitexin, quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-β-D-galactopyranoside, and vitexin-2”-O-rhamnoside), three triterpenes (α-amirin, β-amirin, and lupeol), four steroids (stigmast-22-en-3β-ol, spinasterol, sitostanol, and β-sitosterol) and an alkane (n-nonacosane). As far as we know, this is the only information regarding the leaf chemical profile of this species. The effect on target species is completely unknown.

In this context, the present study was conducted to investigate leaf phytochemicals of *S. polyphylla* and test crude extract and partition phases effect on germination, initial growth, photosynthetic pigments and antioxidant exzymes activities of two target plants (lettuce and tomato).

2.2 Material and method

2.2.1 Sample collection

Senegalia polyphylla (DC.) Britton & Rose branches were collected in São Carlos, São Paulo, Brazil, in May 2022, being identified by Prof. Dr Leonardo Borges (Federal University of São Carlos – UFSCar). A voucher material (LM Borges 1253) is available at SPSC Herbarium.

2.2.2 Crude extract and partition phases preparation

The leaves were dried, powdered, and the crude extract (CE) prepared following the same procedure described in Chapter 1 (pages 21).

Furthermore, an aliquot of CE (20 g) was partitioned as described before, yielding the following partition phases: hexane (Hx = 2.17 g), dichloromethane (DCM = 1.44 g), butanol (BuOH = 4.69 g), ethyl acetate (EtOAc = 0.68 g) and hydroalcoholic residue (EtOH = 9.76 g). A unsoluble residue precipitated during the partition, mainly at hexane, dichloromethane and butanol and partition phases.

2.2.3 Chemical analyses

CE and partition phases were analyzed through HPLC-DAD (CE, BuOH, EtOAc, and EtOH) and GC-MS (Hx and DCM) following the same procedures described in Chapter 1. Compounds suggestion was based on mass spectrum match of the sample and the NYST library (version 2.4 – GC-MS) and through retention time of HPLC-DAD comparison with HPLC-MS-MS data obtained for stem extract (see detailed description in Chapter 1, pages 21-22).

To verify the effect of the CE and partition phases, except EtOAc, on the metabolism of target plants, both, tomato and lettuce seedlings were investigated to photosynthetic pigments content and antioxidant enzyme activities. All analyses were based on absorbance readings using ELISA reader Ultrospec^{TM} 7000 (Chapter 1, pages 24-26).

2.2.4 Coleoptile, germination, and seedling initial growth bioassays

CE and partition phases were prepared and used in the bioassays employing the detailed methodology described in Chapter 1 (pages 23). Coleoptile, shoot, and root length were measured using ImageJ software (version 7.0). As well as for stem, the Hx and DCM partition phases were pooled together to all bioassays, except coleoptile elongation. In the same way, EtOAc partition phase was used only in this first investigation.

2.2.5 Statistical analysis

The data was statistically assessed by one-way analysis of variance (ANOVA) using SPSS v. 21.0 software package. Mean separations were performed by Post hoc Duncan's multiple range tests. Differences at $p < 0.05$ were considered significant.

2.3 Results

2.3.1 Chemical profile of leaf extract and partition phases

The GC-MS profile of *S. polyphylla* leaves using apolar partition phases Hx and DCM revealed the presence of six different compounds (Table 1). These compounds belong to various classes, including terpenes (neophytadiene, squalene, lup-20(29)-3-en-3one, and lupeol) and ester derivatives (hexanedioic acid ethyl ester and octadecanoic acid ethyl ester).

Table 1. The GC-MS suggested compounds in the leaves Hx/DCM partitions phases.

No.	Name of compounds	RT (min)	Chemical formula	Area (%)	Classification
1	Neophytadiene	22.93	$C_{20}H_{38}$	3.75	Terpene
2	Hexanedioic acid ethyl ester	26.00	$C_{18}H_{36}O_2$	12.22	Organic acid ester
3	Octadecanoic acid ethyl ester	29.66	$C_{20}H_{40}O_2$	5.83	Faty acid ester
4	Squalene	39.50	$C_{30}H_5O$	3.55	Triterpene
5	Lup-20(29)-3-en-3one	46.55	$C_{29}H_{48}O$	10.06	Triterpenoid
6	Lupeol	47.17	$C_{30}H_{50}O$	6.16	Triterpenoid

Here, RT = retention time (minutes).

The characterization of polar compounds in the *Senegalia polyphylla* leaves crude extract (CE) and butanol (BuOH) and ethyl acetate (EtOAc) partition phases, and including, and hydroethnolic residue (EtOH) showed various flavonoids derivatives: 3'-methoxypuerarin, herbacetin-3,8-diglucopyranoside, 3'-hydroxypuerarin, 2''-O-beta-L-galactopyranosylorientin, luteolin-6-C-glucoside, rutin, kaempferol 3-O-rutinoside, pectolinarin, 3',4',5,7-tetrahydroxyflavone and 6,4'-dimethoxy-3-hydroxyflavone. The distribution of these compounds in the partition phases are in Table 1S.

2.3.2 Coleoptile bioassay

The *Senegalia polyphylla* leaves crude extract (CE) and its partition phases showed significant inhibitory impact on wheat coleoptile elongation (Figure 1). Generally, all samples, except EtOAc, demonstrated a significant inhibitory effect at all concentrations. As compared to the negative control, maximum inhibition was observed for Hx partition phase that resulted in 42.80% inhibition, followed by the

DCM, BuOH, CE, EtOH and EtOAc, causing 36.41%, 36.40%, 26.35%, 21.75% and 16.42% inhibition at 0.8 mg.mL^{-1}, respectively.

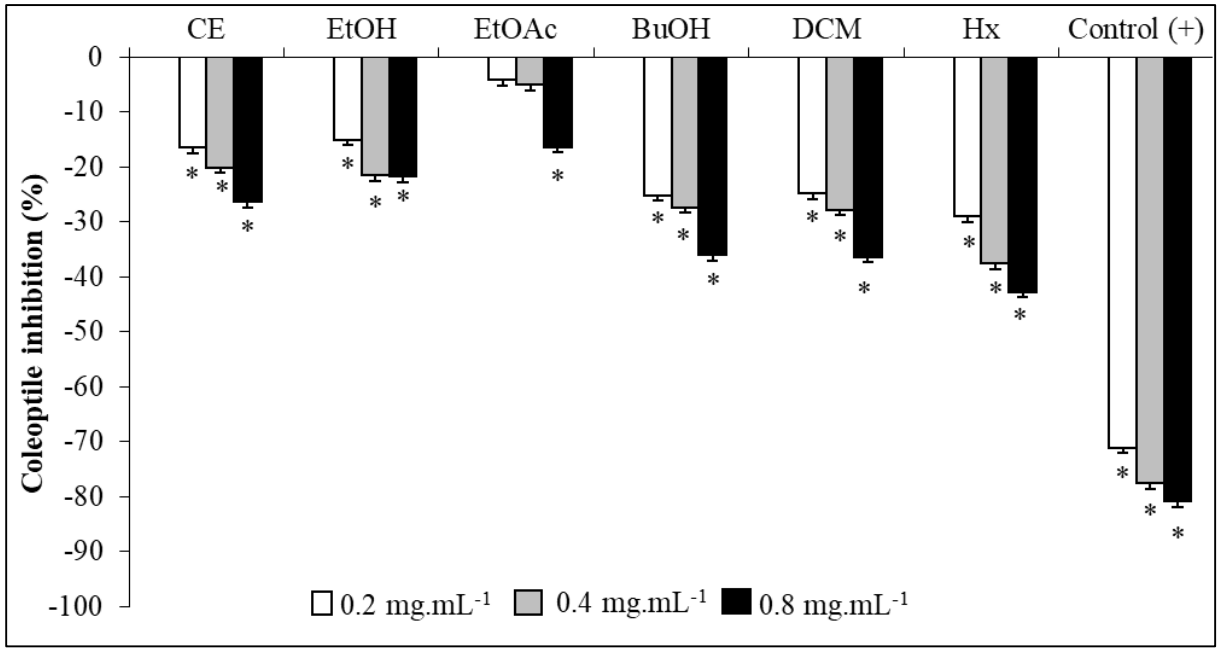


Figure 1. Inhibition percentage of coleoptile elongation of etiolated wheat. Different treatments, like crude extract (CE) and partition phases (Hx = hexane, DCM = dichloromethane, EtOAc = ethyl acetate, BuOH = butanol and EtOH = hydroalcoholic residue) of *Senegalia polyphylla* leaves and herbicide glyphosate (Control +) were implied in this bioassay. Data are mean ± SE of n=15 biological replicates. Asterisks indicate significant statistical differences from negative control at $p \leq 0.05$.

2.3.3 Germination and seedling initial growth bioassay

Based on the similarity of Hx and DCM partition phases unveiled by GC-MS analysis, and the effect on coleoptile elongation bioassay, these partition phases were pooled together for onward bioassays. Additionally, since EtOAc partition phase presented significant effect only with the higher concentration, this sample was not considered for the upcoming bioassays.

The *S. polyphylla* leaves crude extract (CE) and its partition phases differentially affected the germination of target species (Figure 2). As compared to control, CE and EtOH samples did not affect the germination rate of the target species, contrasting with the results of Hx/DCM and BuOH partition phases which inhibited, at 0.8 mg.mL^{-1}, the germination of lettuce by 50.21% and 41.53% and by 65.16% and 56.52% in tomato, respectively.

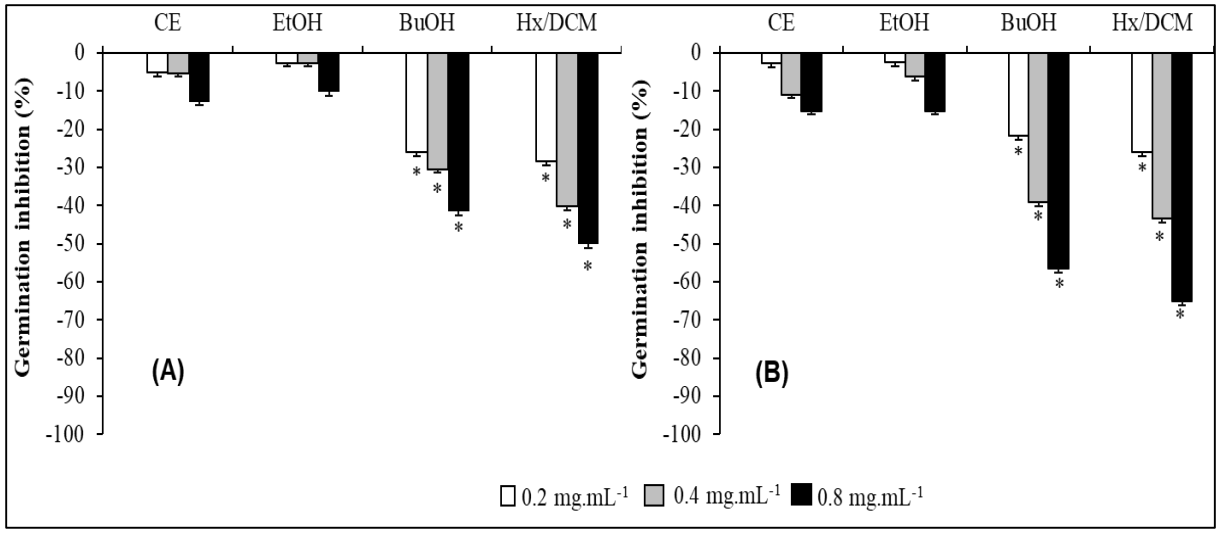


Figure 2. Effect of leaves crude extract (CE) and partition phases (Hx/DCM = hexane + dichloromethane, BuOH = butanol, EtOH = hydroalcoholic residue) of *Senegalia polyphylla* on the germination of lettuce (A) and tomato (B). Data are the mean ± SE of 50 biological replicates. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

The phytotoxic effect of *S. polyphylla* leaves CE and its partitions phases Hx/DCM, BuOH and EtOH on the initial growth of target species was assessed (Figure 3). As well as for germination inhibition, compared to control, CE and EtOH did not affect the root and shoot length of the target species. However, significant dose dependent inhibition was observed for Hx/DCM and BuOH, with tomatoes being more sensitive than lettuce.

Lettuce root and shoot lengths (Figure 3 A and B) were similarly inhibited by Hx/DCM in all concentrations, varying from around 20% - 22% reduction with 0.2 $mg.mL^{-1}$ to around 55% - 60% with 0.8 $mg.mL^{-1}$. Similar effect was observed for BuOH treated seedlings, although in a lower intensity. In this treatment, the higher inhibition observed with 0.8 $mg.mL^{-1}$ of root and shoot lengths were of 40% and 35%, respectively.

Similar pattern was observed for tomato (Figure 3 D and E), even though the effect of Hx/DCM and BuOH partition phases was stronger in this species than in lettuce. The reduction in root length reached 76.52% and 62.57% in Hx/DCM and BuOH 0.8 $mg.mL^{-1}$ treated seedlings, respectively, while the correspondent shoot inhibition was of 73.04% and 50.17%.

The root and shoot reduction observed for both target species, lettuce and tomato, lead to significant reduction in fresh mass of Hx/DCM and BuOH treated seedlings (Figure 3 C and F). As expected, the fresh mass decrease was dose dependent and slightly more pronounced in tomato. Maximum reduction in the fresh biomass of lettuce seedlings was caused by Hx/DCM (52.94%) and BuOH (43.18%) at 0.8 mg.mL^{-1}, while for tomato seedlings, the values were 64.89% and 48.56%, respectively.

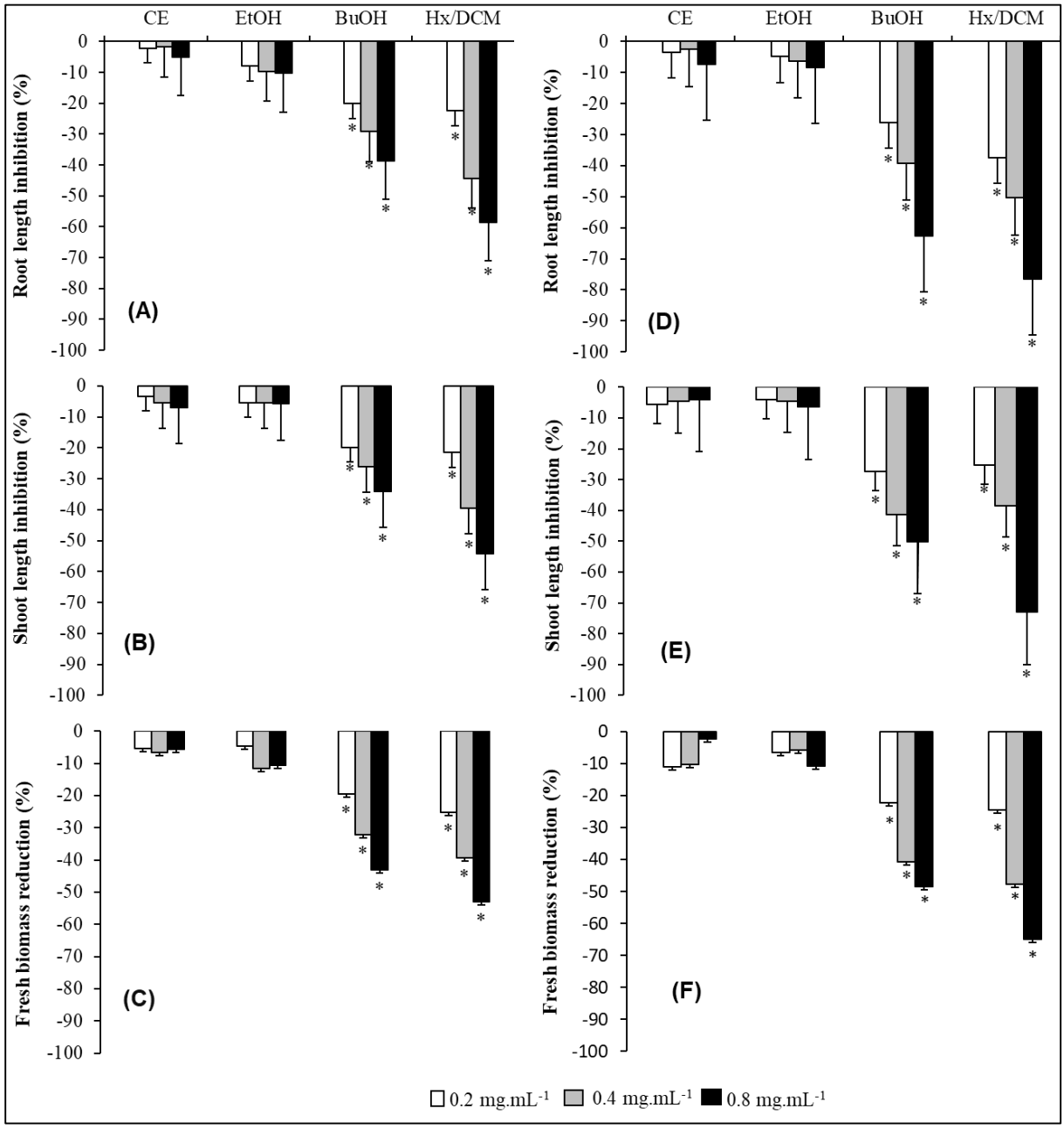


Figure 3. Effect of crude extract (CE) and its partition phases hexane/dichloromethane (Hx/DCM), butanol (BuOH) and ethanol residue (EtOH) on the root and shoot length and fresh biomass of lettuce (A, B and C) and tomato (D, E and F). Data are the mean

± SE of the germinated seedlings. Asterisks represent statistically significant differences from control at $p \leq 0.05$.

Due to non-significant effect of CE and EtOH partition phase in the germination and initial seedling growth, further photosynthetic pigments and antioxidant enzymes analysis were carried out only in the seedlings grown in Hx/DCM and BuOH treatments.

2.3.4 Photosynthetic pigments

Chlorophyll *a*, *b* and carotenoids contents were affected by BuOH and Hx/DCM partition phases (Figure 4).

BuOH partition phase lead to significantly reduction of all photosynthetic pigments in lettuce (Figure 4 A) and tomato (Figure 4 C) seedlings, with exception of chlorophyll *b* of tomato treated with 0.4 $mg.mL^{-1}$, without any correlation with the concentration. For lettuce, chlorophyll *a* and carotenoids reduction were around 50%, while for tomato these values were around 35% and 45%, respectively. However, chlorophyll *b* content presented severe reduction in lettuce, reaching more than 80% in all concentrations.

Despite the reduction of all photosynthetic pigments in the target plants treated with Hx/DCM partition phase, the effect of the higher concentration (0.8 $mg.mL^{-1}$) was more severe (Figure 4 B and D). Again, only chlorophyll *b* of tomato did not present significant reduction with the less concentrated treatments, while for lettuce this pigment reached the greatest reductions ranging from 70% to 85%.

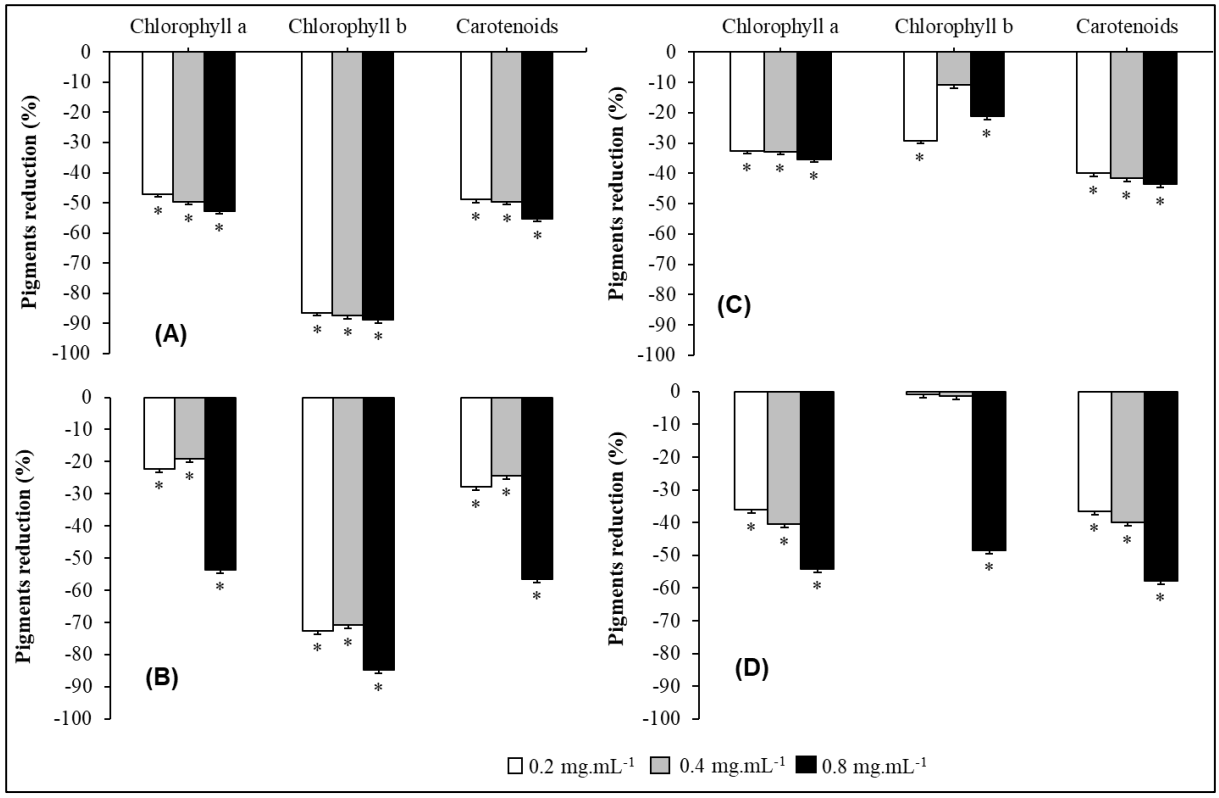


Figure 4. Effect of BuOH (butanol) and Hx/DCM (hexane/dichloromethane) partition phases of *Senegalia polyphylla* on the photosynthetic pigments of lettuce (A and B) and tomato (C and D). Top graphs correspond to BuOH partition phase (A and C), while the bottom ones to Hx/DCM (B and D). Data are means ± SE of three biological replicates. Asterisks indicate significant statistical differences from control at $p \leq 0.05$.

2.3.5 Antioxidant enzymes

The antioxidant enzymes (SOD, CAT and APX) of the target species were differentially affected by Hx/DCM and BuOH partitions phases (Table 2). As compared to control, superoxide dismutase (SOD) activity was significantly stimulated in target species (lettuce and tomato) at all concentrations. This increased activity was very expressive for the two target species grown under the higher concentration (0.8 $mg.mL^{-1}$) of both , Hx/DCM and BuOH, partition phases. . As compared to control, catalase (CAT) activity was not affected in lettuce and tomato seedlings exposed to Hx/DCM at all concentrations. In BuOH grown seedlings, increased activity was observed only in lettuce at 0.2 $mg.mL^{-1}$, as well as for tomato at 0.4 $mg.mL^{-1}$. Lettuce seedlings under Hx/DCM and BuOH treatments did not show significat changes in ascorbate peroxidase (APX) activity, except for seedlings grown with 0.4 $mg.mL^{-1}$ of BuOH partition phase. In an opposite way, in tomato seedlings, APX activity was

upregulated in all tretatments, remaining similar in control only at 0.2 $mg.mL^{-1}$ Hx/DCM.

Table 2. The effect of BuOH (butanol) and Hx/DCM (hexane+dichloromethane) partiton phases on antioxidant enzymes (SOD, CAT and APX) activity in lettuce and tomato seedlings.

Target plants	**Treatments**		**SOD** ($U.mg^{-1}$ of protein)	**CAT** ($\mu mol\ H_2O_2.\ min^{-1}.\ mg^{-1}$ of protein)	**APX** ($\mu mol\ H_2O_2.\ min^{-1}.\ mg^{-1}$ of protein)
Lettuce	Hx/DCM	Control	26.16 ± 0.32	2971.70 ± 6.52	13.21 ± 0.42
		0.2 $mg.mL^{-1}$	110.92 ± 0.81*	1223 ± 7.11	7.47 ± 0.38
		0.4 $mg.mL^{-1}$	305.45 ± 0.92*	687.42 ± 4.19	3.70 ± 0.19
		0.8 $mg.mL^{-1}$	2780.76 ± 1.92*	662.11 ± 5.81	5.54 ± 0.34
	BuOH	Control	22.24 ± 0.32	4659.70 ± 3.15	31.39 ± 0.50
		0.2 $mg.mL^{-1}$	36.63 ± 0.721*	5821.53 ± 9.42*	42.24 ± 0.91*
		0.4 $mg.mL^{-1}$	101.14 ± 2.52*	2462.03 ± 5.95	19.03 ± 0.38
		0.8 $mg.mL^{-1}$	155.04 ± 1.48*	2,029.29 ± 3.17	21.60 ± 0.41
Tomato	Hx/DCM	Control	55.53 ± 1.42	1285.02 ± 7.62	4.80 ± 0.29
		0.2 $mg.mL^{-1}$	140.91 ± 1.82*	965.98 ± 5.19	4.23 ± 0.17
		0.4 $mg.mL^{-1}$	217.40 ± 3.91*	995.06 ± 8.31	6.03 ± 0.22*
		0.8 $mg.mL^{-1}$	2451.03 ± 3.86*	755.50 ± 10.51	5.29 ± 0.48*
	BuOH	Control	61.65 ± 2.15	1673.58 ± 9.21	2.24 ± 0.16
		0.2 $mg.mL^{-1}$	182.45 ± 1.96*	1169.61 ± 10.41	5.87 ± 0.37*
		0.4 $mg.mL^{-1}$	101.65 ± 2.71*	2448.07 ± 13.90*	12.66 ± 0.29*
		0.8 $mg.mL^{-1}$	218.68 ± 5.41*	1433.79 ± 7.18	9.68 ± 0.19*

Where, BuOH (butanol), Hx/DCM (hexane/dichloromethane), SOD (superoxide dismutase), CAT (catalase) and APX (ascorbate peroxidase). Asterisks are used for the statistically significant differences from control at $p \leq 0.05$.

2.4 Discussion

Allelochemicals are phytochemicals that have no primary role in plants, however stimulate/reduces the growth and development of nearly associated target species (Damasceno et al., 2020). In the present study compounds like, terpenes, flavonoids, ester and fatty acid derivatives are suggested as phytochemicals of *Senegalia polyphylla* leaves. These findings are in line with Cesarino *et al*. (2020) who also identified several flavonoids, lipids, triterpenes, steroids and alkane derivatives in the leaf extract of *Acacia polyphylla* (= *Senegalia polyphylla*). On the same way, other groups had also reported phytochemicals belonging to lipids, flavonoids, terpenoids

and alkaloids etc. from the leaves of other *Acacia* species (Jame, 2018; Galib *et al*., 2017; Chaki *et al*., 2015).

Allelopathy, an ecological phenomenon, has long been recognized in the agriculture sector as a cost-effective alternative to traditional herbicides and weedicides (Cheema *et al*., 2012). This process involves the release of allelochemicals, which can either stimulate and/or inhibit seeds germination, growth and development of target species (Macías *et al*., 2003; Zeng *et al*., 2008). In the current study, the phytotoxic effect of *S. polyphylla* leaves crude extract (CE) and its partition phases i.e. hexane/dichloromethane (Hx/DCM), butanol (BuOH), ethyl acetate (EtOAc) and ethanol (EtOH) were checked on wheat coleoptile elongation and seedlings bioassay. Results showed CE and all partition phases (except EtOAc) significantly inhibited coleoptile elongation at all concentrations, as verified by other authors (Matsumoto *et al*., 2014; Rial *et al*., 2014). However, it is worth mentioning that this is the first study using coleoptile approach to address the phytotoxicity of *Senegalia* (or *Acacia*) species.

Curiously, despite coleoptile results, CE and EtOH partition phase did not present significant effect on seed germination and initial growth parameters, including root and shoot length and fresh biomass. The reason for this disparity could be related to the difference between the two systems. While in coleoptile bioassay the compounds act in less complex tissue with less differentiated cells, germination and initial growth assays are developed on more complex systems. Cutler *et al*. (2000) pointed out that coleoptile is more sensitive to a wide range of extracts and bioactive substances, whilst in germination/initial growth bioassays the compounds action depends on seed coat size and permeability (Hanley & Whiting, 2005) and nature of target species (Keating, 1999). This species-specific response to extracts/allelochemicals can shape plant composition in natural ecosystems and convey an interesting idea to design/development of selective herbicides for/in agriculture system (Imatomi *et al*., 2013). Various groups had already reported the phytotoxic effect of extracts and/or phytochemicals obtained from *Acacia* species on target plants, crops and weeds (Chou *et al*., 1998; Lorenzo *et al*., 2011; Jelassi *et al*., 2016) with changes on initial development, as well as on metabolic process like respiration and photosynthesis. Ayeb *et al*. (2013) found significant inhibition of germination and root and shoot development of crop plants (*Triticum aestivum* L. and *Lactuca sativa* L.) and weeds (*Peganum harmala* L. and *Silybum marianum (L.) Gaertn*) submitted to aqueous and

organic extracts from root, stems, phyllodes, flowers, legumes and seeds of *Acacia cyanophylla* Lindl.

Although CE did not significantly affect germination and initial development of lettuce and tomato, BuOH and Hx/DCM partition phases were very active reducing the germination rate, initial development and also the photosynthetic pigments of treated seedlings. Interestingly, phytochemicals detected in Hx/DCM partition phase i.e. hexanedioic acid ethyl ester, linoleic acid, squalene, stigmasterol and lupeol have already being reported for their phytotoxic effect by several researchers (Shen *et al*., 2022; Peguero *et al*., 2012; Ohsawa & Nakatani, 2005; Aguilera *et al*., 2015). Similarly, flavonoids detected in BuOH partition phase have also been previously reported for their potential allelopathic effects (Macías *et al*., 1996; Spring *et al*., 1992; Qiao *et al*., 2021; Johnson *et al*., 2000).

Biotic and abiotic stresses trigger various physiological responses that can impact the efficiency of photosynthesis like, reduced chlorophyll *a*, *b* and carotenoids contents (Al-Johani *et al*., 2012; Šoln *et al*., 2022). In the present study, Hx/DCM and BuOH partition phases of leaf extract of *S. polyphylla* significantly reduced the chlorophyll *a*, *b* and carotenoids in the lettuce and tomato seedlings with a dose dependent effect for Hx/DCM partition phase. Haithloul *et al*. (2022) also detected negative influence of dry leachates of *Acacia saligna* on chlorophyll contents of *Raphanus sativus* L., *Triticum aestivum* L., *Eruca vesicaria* (L.) Cav. and *Hordeum vulgare* L. The reduction in the chlorophyll contents in the target species might be due to the presence of some stressful conditions (Giannakoula *et al*., 2021; Queiroz & Maricle, 2020). The observed reduction in photosynthetic pigments may be linked to the stunted growth of our target seedlings. Since photosynthetic pigments are vital for converting light into other energy form accessible for growth and development (Benavente-Valdés *et al*., 2016), the compromised photosynthetic capacity likely limited energy availability for crucial growth processes, resulting in reduced root and shoot lengths as well as overall fresh weight (Einhellig, 1995; El-Sheekh *et al*., 2010).

Under stressed conditions, excessive ROS generation takes place and, in order to avoid damage, plants up-regulate tolerance mechanisms like antioxidant enzymes (SOD, CAT and APX) designed to eliminate excess ROS (Gautam *et al*., 2016). In this study, SOD contents exhibited significant upregulation across all concentrations and plant species, with a particular hard effect with Hx/DCM partition phase at 0.8 $mg.mL^{-}$

[1]. The upregulation of SOD enzyme holds particular significance, as it represents the first defense line against intracellular reactive oxygen species (ROS) in plants (Ahmad *et al*., 2008), inactivating superoxide radicals and H_2O_2 which can cause oxidative damage to cellular components (Bhattacharya, 2015). Contrasting, CAT activity was not significantly affected in target plants, except for the increased activity at the lower concentrations of BuOH treatment in both, lettuce and tomato seedlings. Additionally, APX activity was up-regulated mainly in tomato seedlings. Catalase (CAT) and ascorbate peroxidase (APX) are integral elements within the plant's antioxidant defense system (Rajput *et al*., 2021). While CAT functions as a swift scavenger decomposing hydrogen peroxide into harmless water and oxygen (Bhattacharya, 2015), APX utilizes ascorbate as an electron donor, effectively neutralizing H_2O_2 and organic hydroperoxides (Hasanuzzaman *et al*., 2020). Zhi-Hui *et al*. (2011) revealed that in lettuce and tomato seedlings grown in allelopathic stress condition, the activity of superoxide dismutase (SOD) significantly increased, while no regulatory expression was observed for peroxidase (POD) and catalase (CAT) contents. Han *et al*. (2013), however, indicated that antioxidant enzymes, including SOD, CAT, and POD, exhibited enhancement at lower treatment concentrations in a phytotoxic assay, but were downregulated at higher concentrations. Further studies, including H_2O_2 quantification and non-enzymatic antioxidants in target plants are crucial to better understand the response mechanism of these plants to allelochemicals.

2.6 Conclusion

Current study demonstrated significant inhibitory effect of *Senegalia polyphylla* leaves crude extract (CE) and its partition phases on growth and development of target species. It was noted that CE and hexane (Hx), dichloromethane (DCM), butanol (BuOH) and hydroethanolic residue (EtOH) partition phases caused inhibition on wheat coleoptile elongation. Hx/DCM and BuOH partition phases presented significant inhibitory effect on germination and growth characters (root and shoot length and reduction in fresh biomass) of lettuce and tomato seedlings, maybe correlated with the significant reduction in chlorophyll *a*, *b*, and carotenoid contents, indicating their possible negative impact on photosynthesis. Furthermore, tomato was much affected than lettuce and Hx/DCM caused more visual effect than BuOH. Additionally, these two treatments also affected SOD activity in both, lettuce and

tomato seedlings, while APX was affected only in the latter. The chemical profiling of the *S. polyphylla* leaves revealed diverse phytochemicals, including, terpenes, carboxylic acids easter, fatty acids, alkaloid and phenolics derivatives. These findings provide valuable insights into the potential allelopathic effects of *S. polyphylla* leaves extract and its partition phases, highlighting their influence on other plants growth, photosynthetic pigments and antioxidant enzymes.

2.7 References

Aguilera, N., Becerra, J., Villaseñor-Parada, C., Lorenzo, P., González, L. and Hernández, V., 2015. Effects and identification of chemical compounds released from the invasive *Acacia dealbata* Link. *Chemistry and Ecology*, *31*(6), pp.479-493.

Ahmad, P., Sarwat, M. and Sharma, S., 2008. Reactive oxygen species, antioxidants and signaling in plants. *Journal of Plant Biology*, *51*, pp.167-173.

ALHaithloul, H.A.S., Khan, M.I., Musa, A., Ghoneim, M.M., ALrashidi, A.A., Khan, I., Azab, E., Gobouri, A.A., Sofy, M.R., El-Sherbiny, M. and Soliman, M.H., 2022. Phytotoxic effects of *Acacia saligna* dry leachates on germination, seedling growth, photosynthetic performance, and gene expression of economically important crops. *PeerJ*, *10*, p.e13623.

Al-Johani, N.S., Aytah, A.A. and Boutraa, T., 2012. Allelopathic impact of two weeds, *Chenopodium murale* and *Malva parviflora* on growth and photosynthesis of barley (*Hordeum vulgare* L.). *Pakistan Journal of Botany*, *44*(6), pp.1865-1872.

Al-Wakeel, S.A.M., Gabr, M.A., Hamid, A.A. and Abu-El-Soud, W.M., 2007. Allelopathic effects of *Acacia nilotica* leaf residue on *Pisum sativum* L. *Allelopathy Journal*, *19*(2), p.411.

AYEB, A.E., Jannet, H.B. and SKHIRI, F.H., 2013. Effects of *Acacia cyanophylla* Lindl. extracts on seed germination and seedling growth of four crop and weed plants. *Turkish Journal of Biology*, *37*(3), pp.305-314.

Benavente-Valdés, J.R., Aguilar, C., Contreras-Esquivel, J.C., Méndez-Zavala, A. and Montañez, J., 2016. Strategies to enhance the production of photosynthetic

pigments and lipids in chlorophycae species. *Biotechnology Reports*, *10*, pp.117-125.

Bhattacharya, S., 2015. Reactive oxygen species and cellular defense system. *Free radicals in human health and disease*, pp.17-29.

Carballeira, A. and Reigosa, M.J., 1999. Effects of natural leachates of *Acacia dealbata* Link in Galicia (NW Spain). *Botanical Bulletin of Academia Sinica*, *40*.

Cesarino, E.C., Gomes, J.D.S., Lessa, E.V., Carvalho Junior, A.R. and Vieira, I.J., 2020. Composição química e atividade antioxidante de *Acacia polyphylla* (Fabaceae). *Revista Virtual de Química*, *12*(2), pp.424-432.

Chaki, S., Ghosh, B., Bandyopadyhay, S., Mookerjee, M., Das, S. and Dastidar, S.G., 2015. Detection of various phytochemical compounds from seeds of *A. auriculiformis* for possibilities of obtaining potent antimicrobial agents. *International Journal of Biology and Pharmacy Research*, *6*(2), pp.120-128.

Cheema, Z.A., Farooq, M. and Wahid, A. eds., 2012. Allelopathy: current trends and future applications. *Springer Science & Business Media*.

Cheng, F. and Cheng, Z., 2015. Research progress on the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopathy. *Frontiers in plant science*, *6*, p.1020.

Chou, C.H., Fu, C.Y., Li, S.Y. and Wang, Y.F., 1998. Allelopathic potential of *Acacia confusa* and related species in Taiwan. *Journal of Chemical Ecology*, *24*, pp.2131-2150.

Cutler, H.G., 1984. A fresh look at the wheat coleoptile bioassay. In *Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America* (pp. 1-9).

Cutler, S.J., Hoagland, R.E. and Cutler, H.G., 2000. Evaluation of selected pharmaceuticals as potential herbicides: bridging the gap between agrochemicals and pharmaceuticals. In *Allelopathy in Ecological Agriculture and Forestry: Proceedings of the III International Congress on Allelopathy in Ecological*

Agriculture and Forestry, Dharwad, India, 18–21 August 1998 (pp. 129-137). Springer Netherlands.

de Brito Damasceno, G.A., Souto, A.L., da Silva, I.B., Roque, A.D.A., Ferrari, M. and Giordani, R.B., 2020. *Prosopis juliflora*: phytochemical, toxicological, and allelochemicals. *Co-evolution of secondary metabolites*, pp.521-541.

de Queiroz, A.R. and Maricle, B.R., 2020. Effects of Low Light on Development of *Ageratina altissima* and *Rudbeckia laciniata* (Asteraceae), Species of the Tallgrass Prairie. *Transactions of the Kansas Academy of Science*, *123*(3-4), pp.387-401.

de Souza Barbosa, R., do Vale, R.S., Schwartz, G., Martins, W.B.R., Ribeiro, S.S., de Matos Rodrigues, J.I., Ferreira, G.C. and Barbosa, V.M., 2022. Restoration of degraded areas after bauxite mining in the eastern Amazon: Which method to apply?. *Ecological Engineering*, *180*, p.106639.

Doyle, J.J. and Luckow, M.A., 2003. The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. *Plant physiology*, 131(3), pp.900-910.

Einhellig, F.A., 1995. Allelopathy: current status and future goals.

El-Khawas, S.A. and Shehata, M.M., 2005. The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. *Biotechnology*, *4*(1), pp.23-34.

El-Sheekh, M.M., Khairy, H.M. and El-Shenody, R.A., 2010. Allelopathic effects of cyanobacterium *Microcystis aeruginosa* Kützing on the growth and photosynthetic pigments of some algal species. *Allelopathy Journal*, *26*(2).

Galib, N.A., Ali, K.S.S., Munaiem, R.T. and Mohammed, A.S., 2017. Phytochemical Screening and thin layer chromatography of *Acacia etbaica* ssp. Uncinata leaves. *World Journal of Pharmaceutical Research*, *6*(2), pp.1278-1283.

Gautam, V., Kaur, R., Kohli, S.K., Verma, V., Kaur, P., Singh, R., Saini, P., Arora, S., Thukral, A.K., Karpets, Y.V. and Kolupaev, Y.E., 2017. ROS compartmentalization in plant cells under abiotic stress condition. *Reactive oxygen*

species and antioxidant systems in plants: role and regulation under abiotic stress, pp.89-114.

Giannakoula, A., Therios, I. and Chatzissavvidis, C., 2021. Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress. *Plants*, *10*(1), p.155.

Han, X., Cheng, Z., Meng, H., Yang, X. and Ahmad, I., 2013. Allelopathic effect of decomposed garlic (*Allium sativum* L.) stalk on lettuce (*L. sativa* var. crispa L.). *Pakistan Journal of Botany*, *45*(1), pp.225-233.

Hanley, M.E. and Whiting, M.D., 2005. Insecticides and arable weeds: effects on germination and seedling growth. *Ecotoxicology*, *14*, pp.483-490.

Hasanuzzaman, M., Bhuyan, M.B., Zulfiqar, F., Raza, A., Mohsin, S.M., Mahmud, J.A., Fujita, M. and Fotopoulos, V., 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. *Antioxidants*, *9*(8), p.681.

Hierro, J.L. and Callaway, R.M., 2003. Allelopathy and exotic plant invasion. *Plant and soil*, *256*, pp.29-39.

Hoque, A.T.M.R., Ahmed, R., Uddin, M.B. and Hossain, M., 2003. Allelopathic effect of different concentration of water extracts of *Acacia auriculiformis* leaf on some initial growth parameters of five common agricultural crops. *Pakistan journal of agronomy*, *2*(2), pp.92-100.

Hussain, M.I., El-Sheikh, M.A. and Reigosa, M.J., 2020. Allelopathic potential of aqueous extract from *Acacia melanoxylon* R. Br. on *Lactuca sativa*. *Plants*, *9*(9), p.1228.

Hussain, W.S. and Abbas, M.M., 2021. Application of allelopathy in crop production. *Agricultural Development in Asia-Potential Use of Nano-Materials and Nano-Technology*.

Imatomi, M., Novaes, P., Matos, A.P., Gualtieri, S.C., Molinillo, J.M., Lacret, R., Varela, R.M. and Macías, F.A., 2013. Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves. *Biochemical Systematics and Ecology*, 46, pp.29-35.

Jame, R., 2018. Phytochemical and pharmacological uses of *Acacia nilotica*—A review. *Seeds*, *1*, pp.15-21.

Jelassi, A., El Ayeb-Zakhama, A., Nejma, A.B., Chaari, A., Harzallah-Skhiri, F. and Jannet, H.B., 2016. Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. *Industrial Crops and Products*, *83*, pp.339-345.

Johnson, G.A., Mantha, S.V. and Day, T.A., 2000. A spectrofluorometric survey of UV-induced blue-green fluorescence in foliage of 35 species. *Journal of Plant Physiology*, *156*(2), pp.242-252.

Kato-Noguchi, H. and Kurniadie, D., 2022. Allelopathy and allelochemicals of *Leucaena leucocephala* as an invasive plant species. *Plants*, *11*(13), p.1672.

Keating, K.I., 1999. Allelopathy: principles, procedures, processes, and promises for biological control. *Advances in agronomy*, 67, pp.141-231.

Li, J., Zhao, T., Chen, L., Chen, H., Luo, D., Chen, C., Miao, Y. and Liu, D., 2022. *Artemisia argyi* allelopathy: a generalist compromises hormone balance, element absorption, and photosynthesis of receptor plants. *BMC Plant Biology*, *22*(1), pp.1-17.

Lima, M.D.R., Ramalho, F.M.G., Trugilho, P.F., Bufalino, L., Júnior, A.F.D., de Paula Protásio, T. and Hein, P.R.G., 2022. Classifying waste wood from Amazonian species by near-infrared spectroscopy (NIRS) to improve charcoal production. *Renewable Energy*, *193*, pp.584-594.

Lorenzo, P., Palomera-Pérez, A., Reigosa, M.J. and González, L., 2011. Allelopathic interference of invasive *Acacia dealbata* Link on the physiological parameters of native understory species. *Plant Ecology*, *212*, pp.403-412.

Macías, F.A., Galindo, J.C. and Molinillo, J.M. eds., 2003. Allelopathy: chemistry and mode of action of allelochemicals. CRC Press.

Macías, F.A., Lacret, R., Varela, R.M., Nogueiras, C. and Molinillo, J.M., 2010. Isolation and phytotoxicity of terpenes from *Tectona grandis*. *Journal of Chemical Ecology*, *36*, pp.396-404.

Macías, F.A., Mejías, F.J. and Molinillo, J.M., 2019. Recent advances in allelopathy for weed control: from knowledge to applications. *Pest management science*, *75*(9), pp.2413-2436.

Macías, F.A., Torres, A., Molinllo, J.G., Varela, R.M. and Castellano, D., 1996. Potential allelopathic sesquiterpene lactones from sunflower leaves. *Phytochemistry*, *43*(6), pp.1205-1215.

Matsumoto, S., Varela, R.M., Palma, M., Molinillo, J.M., Lima, I.S., Barroso, C.G. and Macías, F.A., 2014. Bio-guided optimization of the ultrasound-assisted extraction of compounds from *Annona glabra* L. leaves using the etiolated wheat coleoptile bioassay. *Ultrasonics sonochemistry*, *21*(4), pp.1578-1584.

Meli, P., Isernhagen, I., Brancalion, P.H., Isernhagen, E.C., Behling, M. and Rodrigues, R.R., 2018. Optimizing seeding density of fast-growing native trees for restoring the Brazilian Atlantic Forest. *Restoration Ecology*, *26*(2), pp.212-219.

Ohsawa, R. and Nakatani, C., 2005. Inter-and intra-cultivar variations in the allelopathic effect of leaf aqueous extract of buckwheat (*Fagopyrum esculentum* Moench) on the growth of lettuce seedling. *Fagopyrum*, *22*, pp.21-24.

Peguero, G., Lanuza, O.R., Savé, R. and Espelta, J.M., 2012. Allelopathic potential of the neotropical dry-forest tree *Acacia pennatula* Benth.: inhibition of seedling establishment exceeds facilitation under tree canopies. *Plant ecology*, *213*, pp.1945-1953.

Qiao, Z., Han, L., Liu, X., Dai, H., Liu, C., Yan, M., Li, W., Han, W., Li, X., Huang, S. and Gao, B., 2021. Extraction, radical scavenging activities, and chemical

composition identification of flavonoids from sunflower (*Helianthus annuus* L.) receptacles. *Molecules*, *26*(2), p.403.

Rajput, V.D., Harish, Singh, R.K., Verma, K.K., Sharma, L., Quiroz-Figueroa, F.R., Meena, M., Gour, V.S., Minkina, T., Sushkova, S. and Mandzhieva, S., 2021. Recent developments in enzymatic antioxidant defence mechanism in plants with special reference to abiotic stress. *Biology*, *10*(4), p.267.

Rial, C., Novaes, P., Varela, R.M., G. Molinillo, J.M. and Macias, F.A., 2014. Phytotoxicity of cardoon (*Cynara cardunculus*) allelochemicals on standard target species and weeds. *Journal of Agricultural and food chemistry*, *62*(28), pp.6699-6706.

Sangeetha, C. and Baskar, P., 2015. Allelopathy in weed management: A critical review. *African Journal of Agricultural Research*, *10*(9), pp.1004-1015.

Shen, S., Ma, G., Xu, G., Li, D., Jin, G., Yang, S., Clements, D.R., Chen, A., Wen, L., Zhang, F. and Ye, M., 2022. Allelochemicals identified from sweet potato (*Ipomoea batatas*) and their allelopathic effects on invasive alien plants. *Frontiers in Plant Science*, *13*, p.823947.

Skinner, E.M., Díaz-Pérez, J.C., Phatak, S.C., Schomberg, H.H. and Vencill, W., 2012. Allelopathic effects of sunnhemp (*Crotalaria juncea* L.) on germination of vegetables and weeds. *Horticulture Science*, *47*(1), pp.138-142.

Šoln, K., Klemenčič, M. and Koce, J.D., 2022. Plant cell responses to allelopathy: From oxidative stress to programmed cell death. *Protoplasma*, pp.1-14.

Spring, O., Rodon, U. and Macias, F.A., 1992. Sesquiterpenes from noncapitate glandular trichomes of *Helianthus annuus*. *Phytochemistry*, *31*(5), pp.1541-1544.

Zeng, R.S., 2008. Allelopathy in Chinese ancient and modern agriculture. *Allelopathy in Sustainable Agriculture and Forestry*, pp.39-59.

Zhi-hui, C., Chun-hui, W., Xue-mei, X. and Khan, M.A., 2011. Allelopathic effects of decomposing garlic stalk on some vegetable crops. *African Journal of Biotechnology*, *10*(69), p.15514.

Zhou, Y.H. and Yu, J.Q., 2006. Allelochemicals and photosynthesis. In Allelopathy: A physiological process with ecological implications . *Dordrecht: Springer Netherlands* (pp. 127-139).

Zohaib, A., ABBAS, T. and TABASSUM, T., 2016. Weeds cause losses in field crops through allelopathy. *Notulae Scientia Biologicae*, *8*(1), pp.47-56.

General discussion and conclusion

Phytochemicals (allelochemicals) quality and quantity vary among different tissues and plant species/clade, making allelopathy a complex and intriguing area to study (Stef *et al*., 2013). The leaves and stem of *Senegalia polyphylla* crude extracts (CE) and partition phases i.e. Hexane (Hx), dichloromethane (DCM), butanol (BuOH), ethyl acetate (EtOAc) and hydroalcoholic residues (EtOH) were prepared following Novaes *et al*. (2016) and its chemical composition and influence on initial growth were checked on target species. *S. polyphylla*, leaves and stem chemical profile showed diverse classes of compounds belonging to lipids, ester, terpenoids, alkaloids and phenolic derivatives. These findings are aligned with previous research on *Acacia polyphylla* (= *Senegalia polyphylla*) (Cesarino *et al*., 2020) and other members of *Acacia/Senegalia* (Hussain, 2019; Kumari *et al*., 2022; Janani & Ahamed, 2020).

Allelopathy, as a sub-discipline of ecology, deals with interaction among plant communities where certain plants release allelochemicals into the environment, influencing (positively/negatively) the germination, growth and development of neighboring plants (Khamare *et al*., 2022). Wheat coleoptile elongaition bioassay is a rapid (24-hours), highly sensitive and quicker tool for selecting active samples for further phytotoxic/allelopathic assays (Matsumoto *et al*., 2014). CE and partition phases (except EtOAc at low concentrations) from leave and stem exert significant inhibition of wheat coleoptile elongation, at all concentrations, our study are in agreement with previous research (Novaes *et al*., 2016; Matsumoto *et al.*, 2014). Comparatively, leaves were more phytotoxic than stem, while Hx partition (stem and leaves) phase was more effective than other treatments applied.

Phytotoxic potential of leave and stem CE and its partition phases (except EtOAc) of *S. polyphylla* indicated that hexane/dichloromethane (Hx/DCM) and butanol (BuOH) partition phases significantly affected the germination rate, root and shoot length and fresh biomass of tomato and lettuce. Many research groups had already reported similar inhibitions caused by *Senegalia/Acacia* on the growth traits of many crops (Lorenzo *et al*., 2008; Jelassi *et al*., 2016; El-Khawas *et al*., 2005; Oyun, 2006). Again, as well as in the coleoptile bioassay, leaves were more phytotoxic than stem. Maybe the amount of the allelopathic compounds was greater, a hypothesis that deserves attention.

The compounds annotated for *S. polyphylla* have already been previously reported for their phytotoxic properties (Shen *et al*., 2022; Peguero *et al*., 2012; Ohsawa & Nakatani, 2005; Aguilera *et al*., 2015; Macías *et al*., 1996; Johnson *et al*., 2000).

Plants exposed to stressful conditions adapt by undergoing alterations in their morphological and anatomical characteristics (Bhargava & Sawant, 2013). In the present study, root length was much affected by stem and leaves partition phases, with hard anatomical changes observed with stem partition phases (Hx/DCM and BuOH) treated seedlings, leading to a deep disruption on root organization in tomato seedlings grown under BuOH partition phase. The formation of aerenchyma tissues may be linked to the reduction in root and shoot biomass observed in our study, as reported in wheat and barley under waterlogging conditions (Celedonio *et al*., 2017) and *Cynodon dactylon* seedlings (Yuna *et al*., 2022).

Any disruption in chlorophyll contents serve as a valuable indicator in assessing the impact of environmental stresses (Roca *et al*., 2016). The significant reduction in chlorophyll *a*, *b* and carotenoid contents in tomato and lettuce seedlings treated with leaves and stem partition phases suggested that the photosynthetic process was affected. Similar findings have been reported by Batish *et al*. (2007), who observed decreased chlorophyll contents in legume species under the influence of allelopathic compounds. Plants exposed to environmental stresses generate an excess of reactive oxygen species (ROS) that can overwhelm their antioxidant defense systems (Karuppanapandian *et al*., 2011). In current study, SOD activity in the target species was increased significantly in response to stem and leaves partition phases (BuOH and Hx/DCM), reinforcing the activation of the "first line" responses of the target plants to the stresseful condition. Deeper investigation is needed to understand the role of other antioxidants enzymes, as well as, some non-enzymatic compounds important to mitigate plant stress.

Further research is recommended to isolate active compound and elucidate the specific mechanisms underlying these effects and their ecological implications. Understanding specific allelopathic interactions between plants can provide valuable insights into natural plant defense mechanisms and potential applications in agriculture and weed management.

References

Aguilera, N., Becerra, J., Villaseñor-Parada, C., Lorenzo, P., González, L. and Hernández, V., 2015. Effects and identification of chemical compounds released from the invasive *Acacia dealbata* Link. *Chemistry and Ecology*, *31*(6), pp.479-493.

Ali, K.A., Sakri, F.Q. and Li, Q.X., 2012. Isolation and purification of allelochemicals from *Cephalaria syriaca* plant. *International Journal of Biosciences*, *2*, pp.90-103.

Batish, D.R., Lavanya, K., Singh, H.P. and Kohli, R.K., 2007. Phenolic allelochemicals released by *Chenopodium murale* affect the growth, nodulation and macromolecule content in chickpea and pea. *Plant Growth Regulation*, 51, pp.119-128.

Bhargava, S. and Sawant, K., 2013. Drought stress adaptation: metabolic adjustment and regulation of gene expression. *Plant breeding*, *132*(1), pp.21-32.

Cesarino, E.C., Gomes, J.D.S., Lessa, E.V., Carvalho Junior, A.R. and Vieira, I.J., 2020. Composição química e atividade antioxidante de *Acacia polyphylla* (Fabaceae). *Revista Virtual de Química*, *12*(2), pp.424-432.

Davis, A.R., Butler, A.J. and van Altena, I., 1991. Settlement behaviour of ascidian larvae: preliminary evidence for inhibition by sponge allelochemicals. *Marine Ecology Progress Series*, pp.117-123.

de San Celedonio, R.P., Abeledo, L.G., Mantese, A.I. and Miralles, D.J., 2017. Differential root and shoot biomass recovery in wheat and barley with transient waterlogging during preflowering. *Plant and Soil*, *417*, pp.481-498.

El-Khawas, S.A. and Shehata, M.M., 2005. The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.) plants. *Biotechnology*, *4*(1), pp.23-34.

Hussain, M.M., 2019. A mini review on the chemical compounds of the genus *Acacia*. *Bangladesh Pharmaceutical Journal*, *22*(2), pp.235-242.

Janani, M. and Ahamed, M.N., 2020. A review on traditional uses, phytochemistry and pharmacological activities of *Acacia nilotica* (Indian babul). *International Journal of Biomedical Nanoscience and Nanotechnology*, *4*(3-4), pp.148-174.

Jelassi, A., El Ayeb-Zakhama, A., Nejma, A.B., Chaari, A., Harzallah-Skhiri, F. and Jannet, H.B., 2016. Phytochemical composition and allelopathic potential of three Tunisian *Acacia* species. *Industrial Crops and Products*, *83*, pp.339-345.

Johnson, G.A., Mantha, S.V. and Day, T.A., 2000. A spectrofluorometric survey of UV-induced blue-green fluorescence in foliage of 35 species. *Journal of Plant Physiology*, *156*(2), pp.242-252.

Karuppanapandian, T., Moon, J.C., Kim, C., Manoharan, K. and Kim, W., 2011. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. *Australian Journal of Crop Science*, *5*(6), pp.709-725.

Khamare, Y., Chen, J. and Marble, S.C., 2022. Allelopathy and its application as a weed management tool: A review. *Frontiers in Plant Science*, *13*, p.1034649.

Kumari, M., Radha, Kumar, M., Zhang, B., Amarowicz, R., Puri, S., Pundir, A., Rathour, S., Kumari, N., Chandran, D. and Dey, A., 2022. *Acacia catechu* (Lf) Willd.: A review on bioactive compounds and their health promoting functionalities. *Plants*, *11*(22), p.3091.

Lorenzo, P., Pazos-Malvido, E., González, L. and Reigosa, M.J., 2008. Allelopathic interference of invasive *Acacia dealbata*: physiological effects. *Allelopathy Journal*, *22*(2), pp.64-76.

Macías, F.A., Torres, A., Molinllo, J.G., Varela, R.M. and Castellano, D., 1996. Potential allelopathic sesquiterpene lactones from sunflower leaves. *Phytochemistry*, *43*(6), pp.1205-1215.

Matsumoto, S., Varela, R.M., Palma, M., Molinillo, J.M., Lima, I.S., Barroso, C.G. and Macías, F.A., 2014. Bio-guided optimization of the ultrasound-assisted extraction of compounds from *Annona glabra* L. leaves using the etiolated wheat coleoptile bioassay. *Ultrasonics sonochemistry*, *21*(4), pp.1578-1584.

Mayworm, M.A., dos Santos, A.B., Melo-de-Pinna, G.F., Salatino, A., Salatino, M.L. and Demarco, D., 2017. Monoterpenes from the essential oil from Brazilian propolis affect seedling cellular elongation. *Brazilian Journal of Botany*, *40*, pp.609-615.

Müller, M. and Munné-Bosch, S., 2021. Hormonal impact on photosynthesis and photoprotection in plants. *Plant Physiology*, 185(4), pp.1500-1522.

Murali, M., Gowtham, H.G., Singh, S.B., Shilpa, N., Aiyaz, M., Alomary, M.N., Alshamrani, M., Salawi, A., Almoshari, Y., Ansari, M.A. and Amruthesh, K.N., 2022. Fate, bioaccumulation and toxicity of engineered nanomaterials in plants: current challenges and future prospects. *Science of The Total Environment*, *811*, p.152249.

Nebo, L., Varela, R.M., Molinillo, J.M., Sampaio, O.M., Severino, V.G., Cazal, C.M., das Graças Fernandes, M.F., Fernandes, J.B. and Macías, F.A., 2014. Phytotoxicity of alkaloids, coumarins and flavonoids isolated from 11 species belonging to the Rutaceae and Meliaceae families. *Phytochemistry Letters*, *8*, pp.226-232.

Novaes, P., Torres, P.B. and dos Santos, D.Y.A.C., 2016. Biological activities of Annonaceae species extracts from Cerrado. *Brazilian Journal of Botany*, *39*, pp.131-137.

Ohsawa, R. and Nakatani, C., 2005. Inter-and intra-cultivar variations in the allelopathic effect of leaf aqueous extract of buckwheat (*Fagopyrum esculentum* Moench) on the growth of lettuce seedling. *Fagopyrum*, *22*, pp.21-24.

Oyun, M.B., 2006. Allelopathic potentialities of *Gliricidia sepium* and *Acacia auriculiformis* on the germination and seedling vigour of maize (*Zea mays* L.). *American Journal of Agricultural and Biological Science*, *1*(3), pp.44-47.

Peguero, G., Lanuza, O.R., Savé, R. and Espelta, J.M., 2012. Allelopathic potential of the neotropical dry-forest tree *Acacia pennatula* Benth.: inhibition of seedling establishment exceeds facilitation under tree canopies. *Plant ecology*, *213*, pp.1945-1953.

Retig, B., Holm, L.G. and Struckmeyer, B.E., 1972. Effects of weeds on the anatomy of roots of cabbage and tomato. *Weed Science*, *20*(1), pp.33-36.

Rial, C., García, B.F., Varela, R.M., Torres, A., Molinillo, J.M. and Macías, F.A., 2016. The joint action of sesquiterpene lactones from leaves as an explanation for the activity of *Cynara cardunculus*. *Journal of agricultural and food chemistry*, *64*(33), pp.6416-6424.

Rial, C., Novaes, P., Varela, R.M., G. Molinillo, J.M. and Macias, F.A., 2014. Phytotoxicity of cardoon (*Cynara cardunculus*) allelochemicals on standard target species and weeds. *Journal of Agricultural and food chemistry*, *62*(28), pp.6699-6706.

Roca, G., Alcoverro, T., Krause-Jensen, D., Balsby, T.J., Van Katwijk, M.M., Marbà, N., Santos, R., Arthur, R., Mascaró, O., Fernández-Torquemada, Y. and Pérez, M., 2016. Response of seagrass indicators to shifts in environmental stressors: a global review and management synthesis. *Ecological Indicators*, *63*, pp.310-323.

Shen, S., Ma, G., Xu, G., Li, D., Jin, G., Yang, S., Clements, D.R., Chen, A., Wen, L., Zhang, F. and Ye, M., 2022. Allelochemicals identified from sweet potato (*Ipomoea batatas*) and their allelopathic effects on invasive alien plants. *Frontiers in Plant Science*, *13*, p.823947.

Stef, R., Bostan, C., Butu, A., Ortan, A., Rodino, S. and Butu, M., 2013. Comparative characteristics of *Lupinus perennis* L. under allelochemical sorgoleone stress. *Romanian Biotechnological Letters*, *18*(3), pp.8327-8332.

Takahashi, H., Yamauchi, T., Colmer, T.D. and Nakazono, M., 2014. Aerenchyma formation in plants. *Low-Oxygen Stress in Plants: Oxygen Sensing and Adaptive Responses to Hypoxia*, pp.247-265.

RESUMO

A alelopatia explora as intrincadas interações químicas entre as plantas, resultando em efeitos favoráveis e/ou adversos em espécies-alvo. Este estudo teve como objetivo investigar a composição química do caule e das folhas de *Senegalia polyphylla* e o impacto dos extratos em espécies-alvo. Extratos brutos (CE) de caule e folhas, as fases de partição Hx (hexano), diclorometano (DCM), butanol (BuOH), acetato de etila (EtOAc) e o resíduo hidroalcoólico (EtOH) foram investigados em bioensaios de análise do alongamento do coleóptilo de trigo e de germinação e crescimento inicial em alface e tomate, nas concentrações de 0,2, 0,4 e 0,8 $mg.mL^{-1}$. Através de análises em CLAE-EM/EM e CG-EM foram detectados derivados de flavonoides, fosfolipídios, glicolipídios, glicerídeos, derivados de alcaloides, terpenos, ácidos graxos e derivados de éster. De modo geral, as plântulas de tomate foram mais sensíveis que as de alface. Apesar de serem observados padrões muito semelhantes, os extratos e fases de partição obtidos das folhas foram mais fitotóxicos que os de caule, assim como os efeitos observados para a fase de partição combinada Hx/DCM foram mais pronunciados que para a fase de partição de BuOH. Tanto o CE do caule como das folhas e as suas fases de partição, com exceção do EtOAc, inibiram significativamente o alongamento do coleóptilo do trigo. Da mesma forma, as fases de partição BuOH e Hx/DCM inibiram significativamente a taxa de germinação, o comprimento da raiz e da parte aérea, levando a redução da biomassa fresca das plântulas de alface e tomate. Além disso, essas fases de partição levaram a redução dos teores dos pigmentos fotossintéticos (clorofila *a*, *b* e carotenoides) nas duas espécies alvo, o que pode estar relacionado ao menor crescimento inicail das plântulas. No entanto, as enzimas antioxidantes foram afetadas diferencialmente. Comparando com o controle, as plântulas de alface e tomate apresentaram maior atividade da SOD em todos os tratamentos, reforçando a ideia de ativação do sistema de defesa da planta contra agentes estressores. Já CAT e APX apresentaram respostas variáveis, não possibilitando entender o papel dessas enzimas nesse contexto. Alterações anatômicas marcantes foram observadas nas raízes das plântulas das espécies alvo submentidas aos tratamentos das fases de partição Hx/DCM e BuOH (0,8 $mg.mL^{-1}$) do extrato de caule da *S. polyphylla*. A análise microscópica revelou a formação de aerênquima, células corticais menores e espaços intercelulares no cortex e no sistema vascular. Assim, este estudo destaca os efeitos fitotóxicos significativos dos extratos de caule e das folhas de

S. polyphylla, alterando morfologica e fisiologicamente as espécies alvo. Recomenda-se mais investigação para isolar os compostos ativos e avaliar o impacto dessa espécie em culturas importantes em condições de campo.

Palavras-chave: alelopatia, fitoquímicos, Senegalia polyphylla, Fabaceae, crescimento inicial, pigmentos fotossintéticossis, extração.

ABSTRACT

Allelopathy addresses the intricate chemical interactions between plants, resulting in favorable and/or adverse effects on target species. This study aimed to investigate the chemical composition of the stem and leaves of *Senegalia polyphylla* and the impact of the extracts on target species. Crude extracts (CE) of stem and leaves, the partition phases Hx (hexane), dichloromethane (DCM), butanol (BuOH), ethyl acetate (EtOAc) and the hydroalcoholic residue (EtOH) were investigated in bioassays of wheat coleoptile elongation and germination and initial growth in lettuce and tomato, at concentrations of 0.2, 0.4 and 0.8 mg.mL^{-1}. Through HPLC-MS/MS and GC-MS analyses, derivatives of flavonoids, phospholipids, glycolipids, glycerides, alkaloid derivatives, terpenes, fatty acids and ester derivatives were detected. In general, tomato seedlings were more sensitive than lettuce seedlings. Despite very similar patterns, the extracts and partition phases obtained from the leaves were more phytotoxic than those from the stem, as well as the effects for the combined Hx/DCM partition phase were more pronounced than for the BuOH partition phase. Both stem and leaf CE and their partition phases (except EtOAc) significantly inhibited wheat coleoptile elongation. Likewise, the BuOH and Hx/DCM partition phases significantly inhibited the germination rate, root and shoot length, leading to a reduction in the fresh biomass, of lettuce and tomato seedlings. Furthermore, these partition phases led to a reduction in the levels of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids) in the two target species, which may be related to the lower initial growth of the seedlings. However, antioxidant enzymes were differentially affected. Compared to the control, lettuce and tomato seedlings showed greater SOD activity in all treatments, reinforcing the idea of activation of the initial plant's defense system against stressors. CAT and APX showed variable responses, making it impossible to understand the role of these enzymes in this context. Marked anatomical changes were observed in the roots of lettuce and tomato seedlings subjected to Hx/DCM and BuOH (0.8 mg.mL^{-1}) partition phases of *S. polyphylla* stem extract. Microscopic analysis revealed the formation of aerenchyma, smaller cortical cells and intercellular spaces in the cortex and vascular cylinder. Thus, this study highlights the significant phytotoxic effects of *S. polyphylla* stem and leaf extracts, reaching morphological and physiological traits of the target species. Further investigation is recommended to isolate the active compounds and evaluate the impact of this species on important crops under field conditions.

Keys words; allelopathy, phytochemicals, *Senegalia polyphylla*, Fabaceae, initial growth, photosynthetic pigments

Appendices

Figure 1. Generalized presentation (Control vs treatments) of lettuce and tomato seedlings exposed to stem derived partitions phases i.e. butanol and hexane/dichloromethane, respectively.

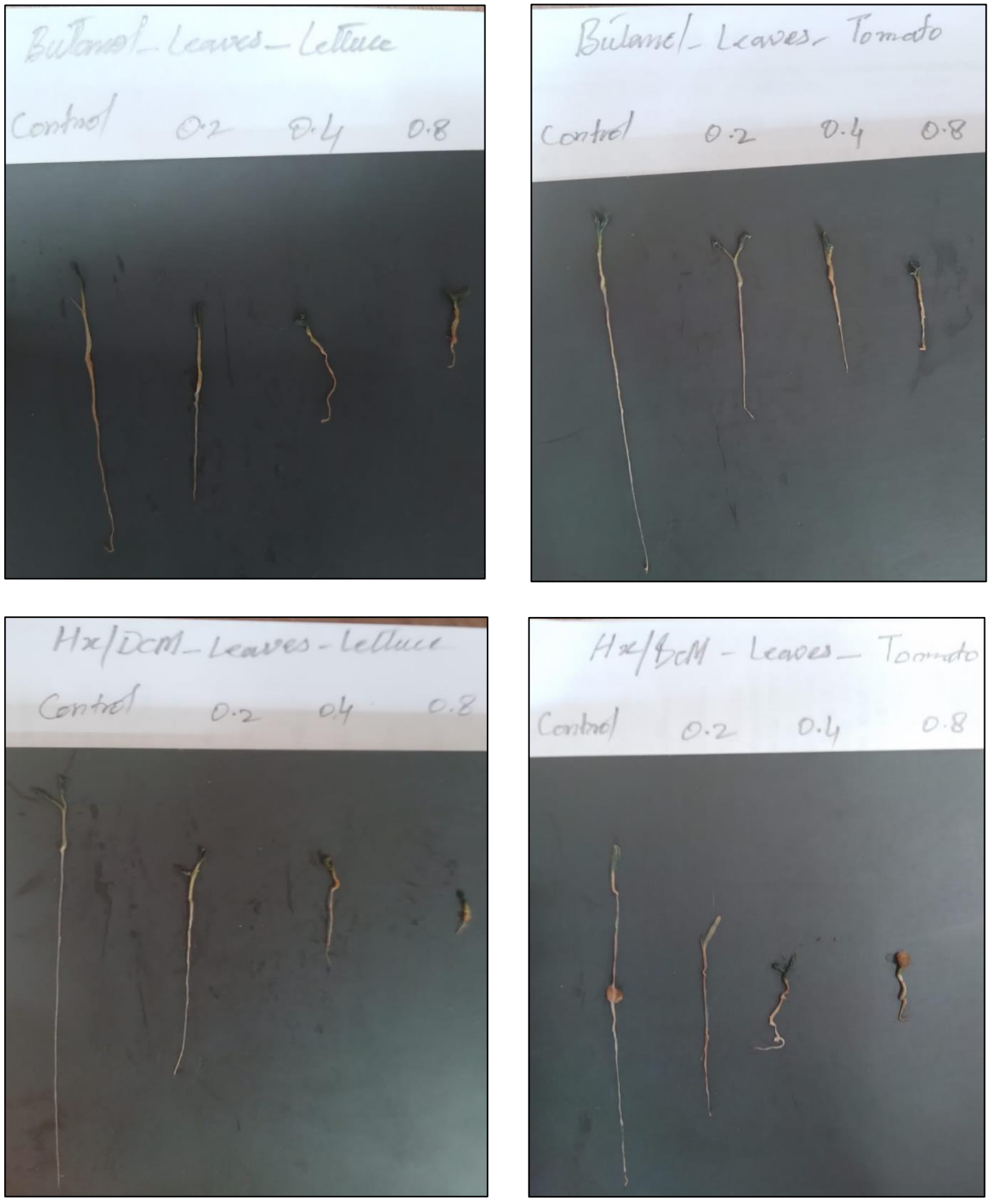
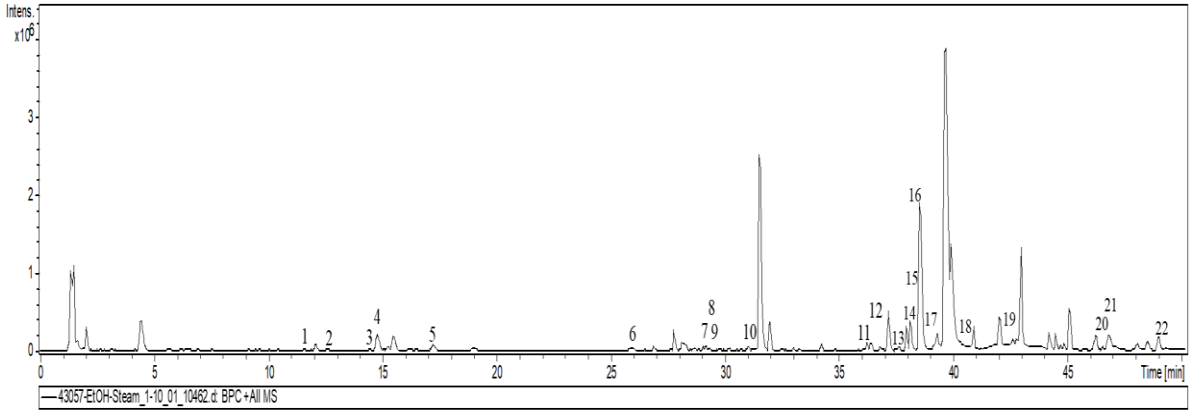
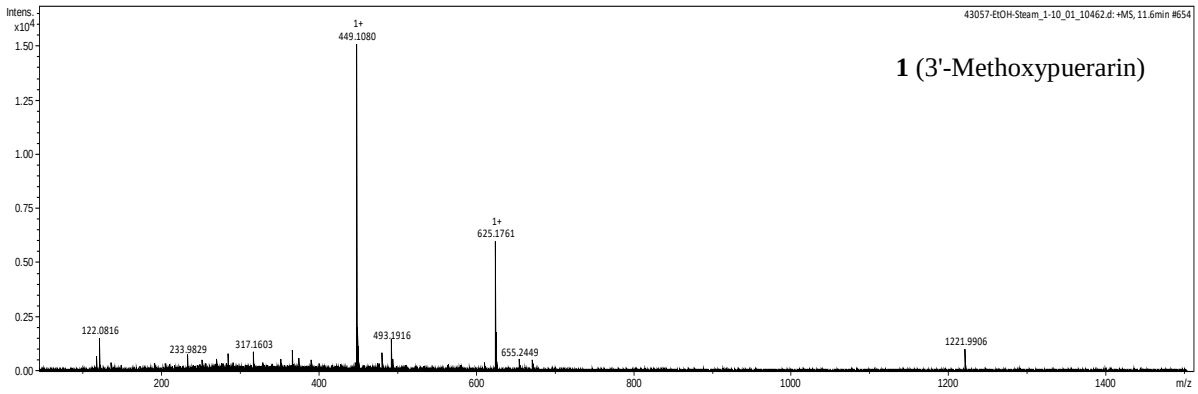


Figure 2. Generalized presentation (Control vs treatments) of lettuce and tomato seedlings exposed to leaves derived partitions phases i.e. butanol and hexane/dichloromethane, respectively.

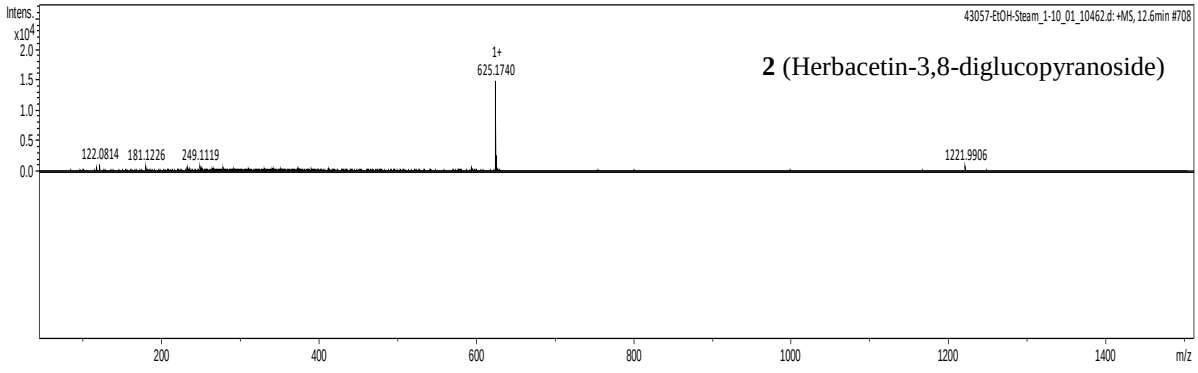
Stem crude extract analyzed by LC-MS-MS

Chromatogram and mass spectra of compounds. The number above each peak in the chromatogram is used in the correspondent mass spectra. Compound name, based on GNPS annotation, is inside parenthesis in the mass spectra.

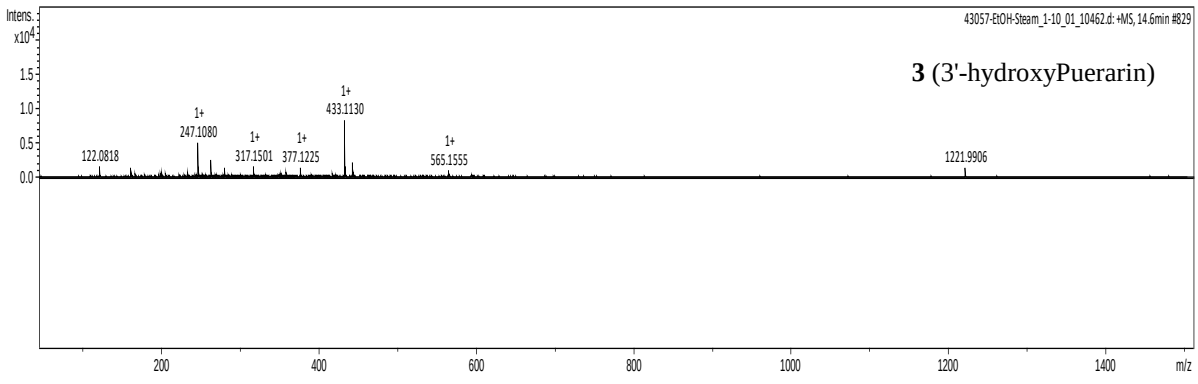




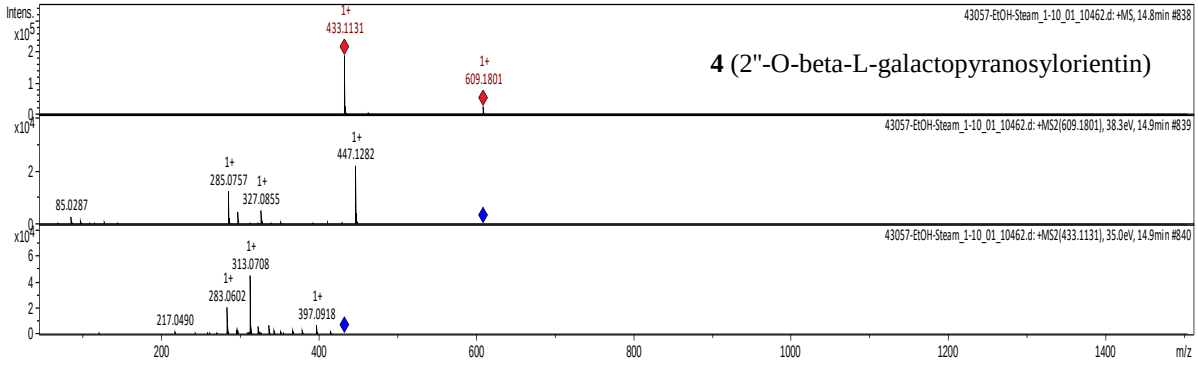
1 (3'-Methoxypuerarin)

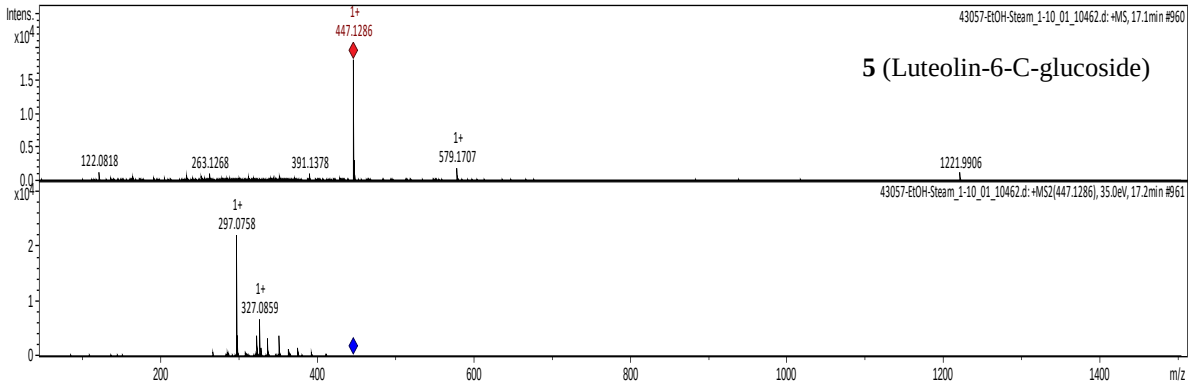


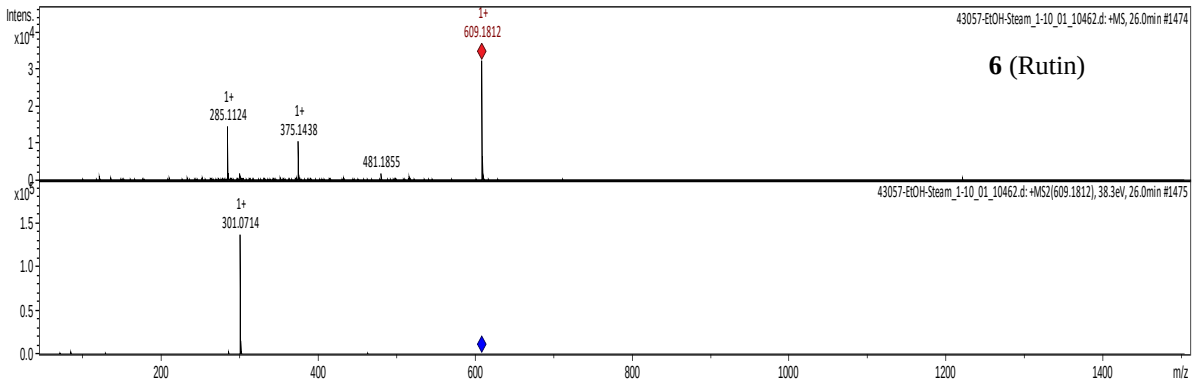
2 (Herbacetin-3,8-diglucopyranoside)



3 (3'-hydroxyPuerarin)


Intens.
1+
433.1131
1+
609.1801
43057-EtOH-Steam_1-10_01_10462.d: +MS, 14.8min #838
4 (2"-O-beta-L-galactopyranosylorientin)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(609.1801), 38.3eV, 14.9min #839
1+
447.1282
1+
285.0757
1+
327.0855
85.0287
43057-EtOH-Steam_1-10_01_10462.d: +MS2(433.1131), 35.0eV, 14.9min #840
1+
313.0708
1+
283.0602
1+
397.0918
217.0490
m/z


Intens.
1+
447.1286
43057-EtOH-Steam_1-10_01_10462.d: +MS, 17.1min #960
5 (Luteolin-6-C-glucoside)
1+
579.1707
122.0818
263.1268
391.1378
1221.9906
43057-EtOH-Steam_1-10_01_10462.d: +MS2(447.1286), 35.0eV, 17.2min #961
1+
297.0758
1+
327.0859
m/z


Intens.
1+
609.1812
43057-EtOH-Steam_1-10_01_10462.d: +MS, 26.0min #1474
6 (Rutin)
1+
285.1124
1+
375.1438
481.1855
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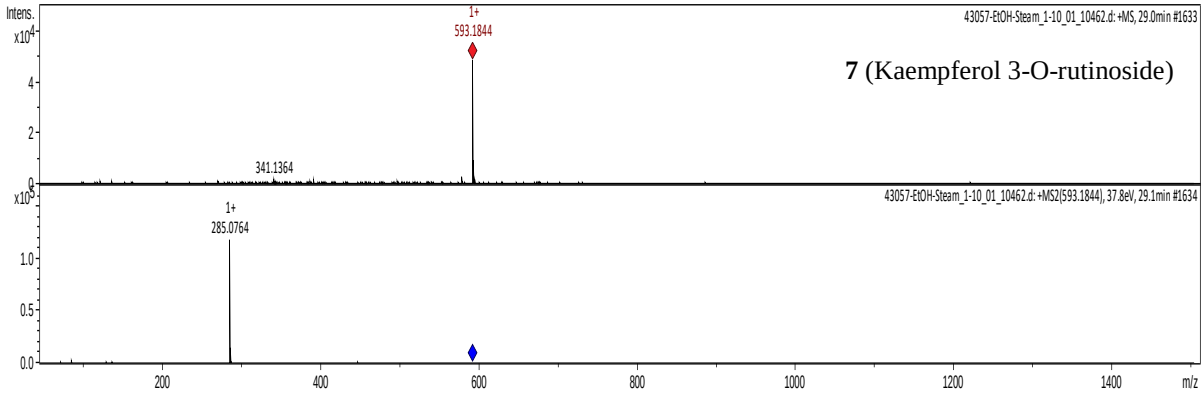
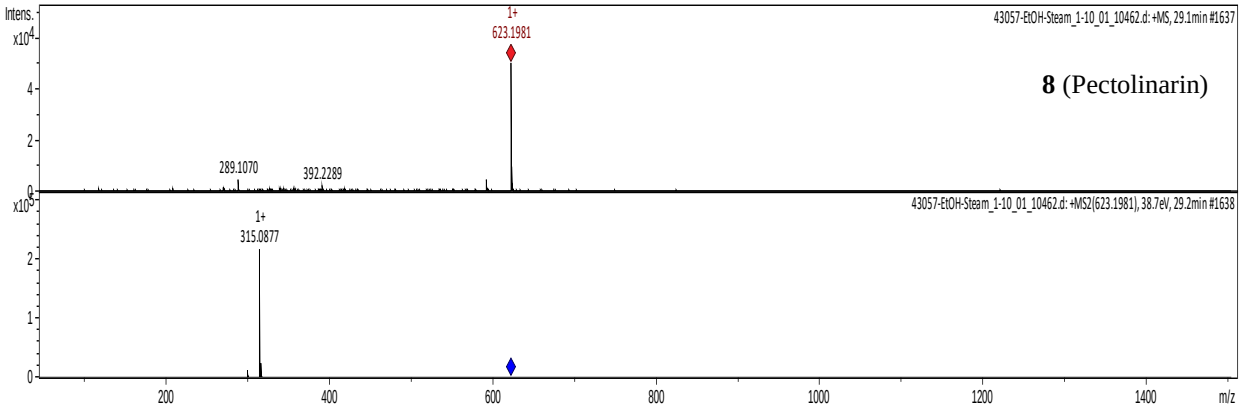
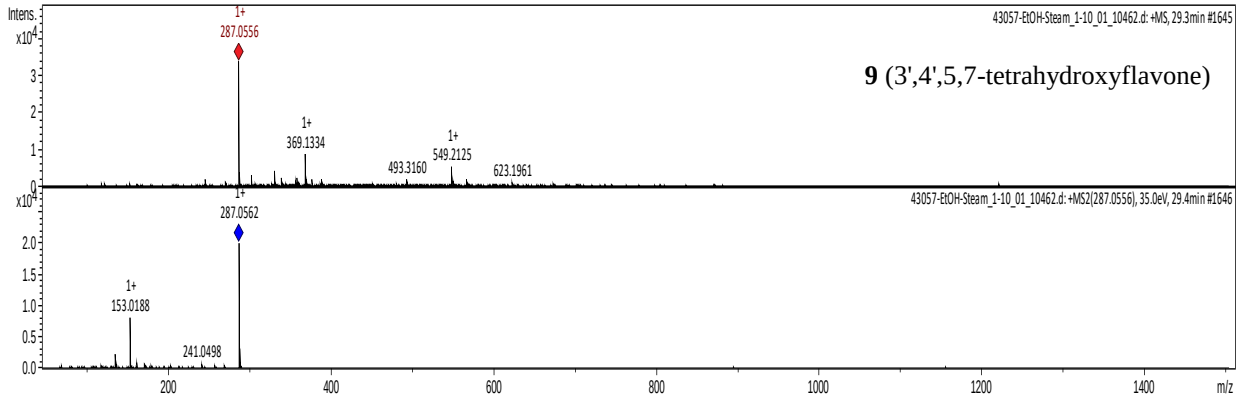
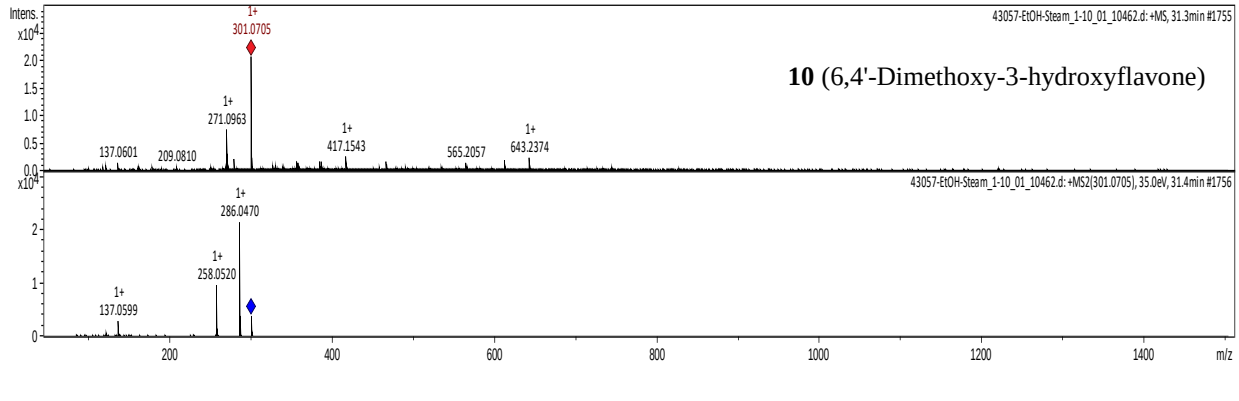
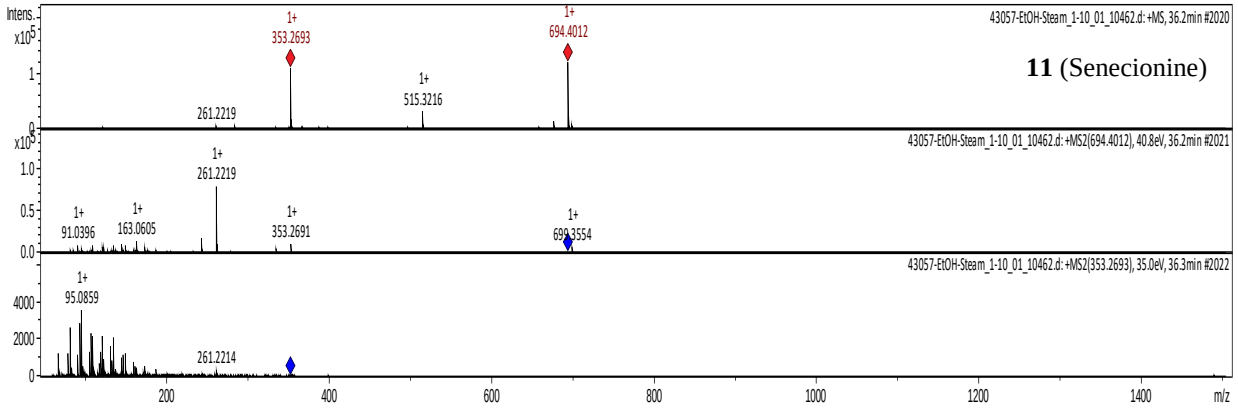
1+
301.0714
m/z

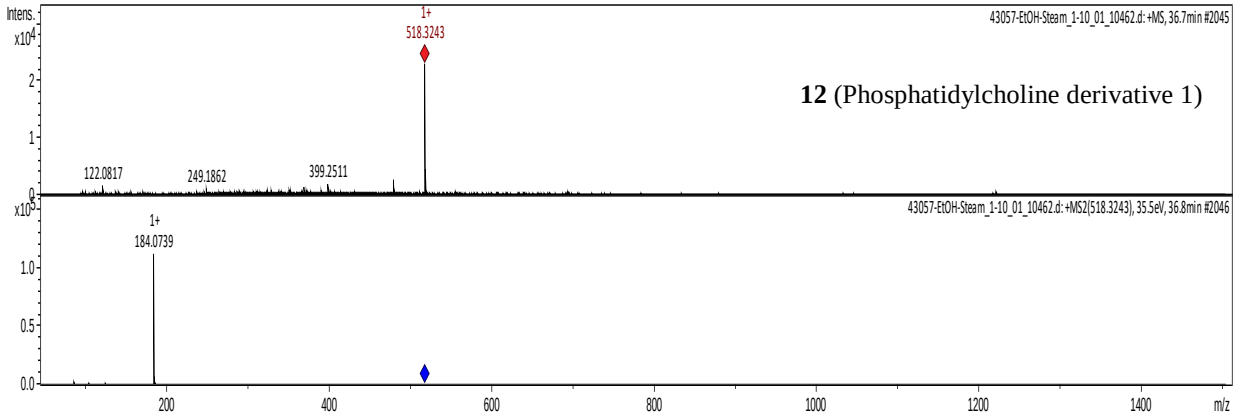

Intens.
1+
593.1844
43057-EtOH-Steam_1-10_01_10462.d: +MS, 29.0min #1633
7 (Kaempferol 3-O-rutinoside)
341.1364
43057-EtOH-Steam_1-10_01_10462.d: +MS2(593.1844), 37.8eV, 29.1min #1634
1+
285.0764
m/z


43057-EtOH-Steam_1-10_01_10462.d: +MS, 29.1min #1637
1+
623.1981
289.1070
392.2289
8 (Pectolinarin)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(623.1981), 38.7eV, 29.2min #1638
1+
315.0877
Intens.
m/z

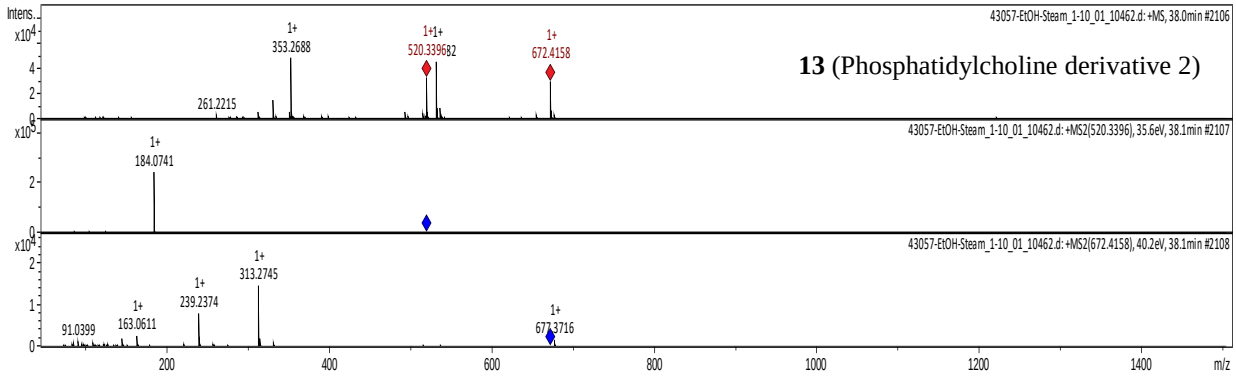

43057-EtOH-Steam_1-10_01_10462.d: +MS, 29.3min #1645
1+
287.0556
1+
369.1334
1+
549.2125
493.3160
623.1961
9 (3',4',5,7-tetrahydroxyflavone)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(287.0556), 35.0eV, 29.4min #1646
1+
287.0562
1+
153.0188
241.0498
Intens.
m/z


43057-EtOH-Steam_1-10_01_10462.d: +MS, 31.3min #1755
1+
301.0705
1+
271.0963
1+
417.1543
1+
643.2374
137.0601
209.0810
565.2057
10 (6,4'-Dimethoxy-3-hydroxyflavone)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(301.0705), 35.0eV, 31.4min #1756
1+
286.0470
1+
258.0520
1+
137.0599
Intens.
m/z

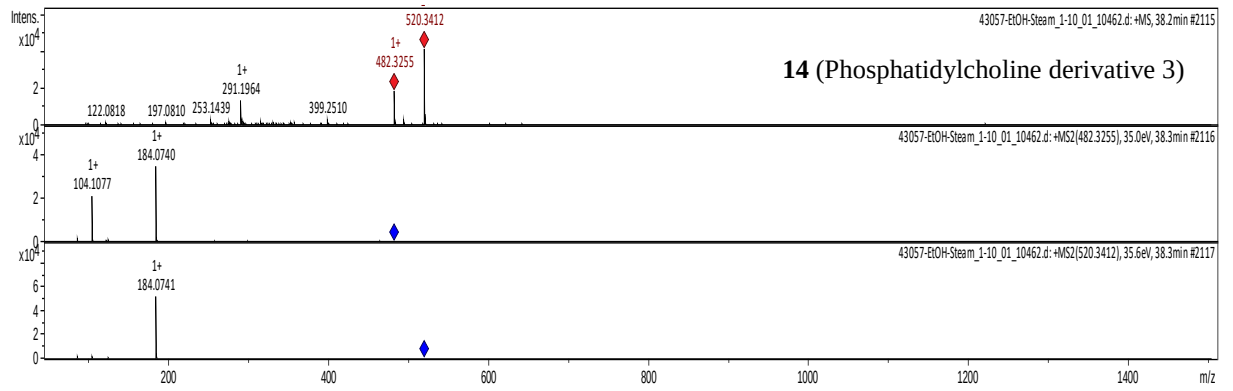

43057-EtOH-Steam_1-10_01_10462.d: +MS, 36.2min #2020
1+
353.2693
1+
694.4012
1+
515.3216
261.2219
11 (Senecionine)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(694.4012), 40.8eV, 36.2min #2021
1+
261.2219
1+
91.0396
1+
163.0605
1+
353.2691
1+
694.3554
43057-EtOH-Steam_1-10_01_10462.d: +MS2(353.2693), 35.0eV, 36.3min #2022
1+
95.0859
261.2214
Intens.
m/z



12 (Phosphatidylcholine derivative 1)



13 (Phosphatidylcholine derivative 2)



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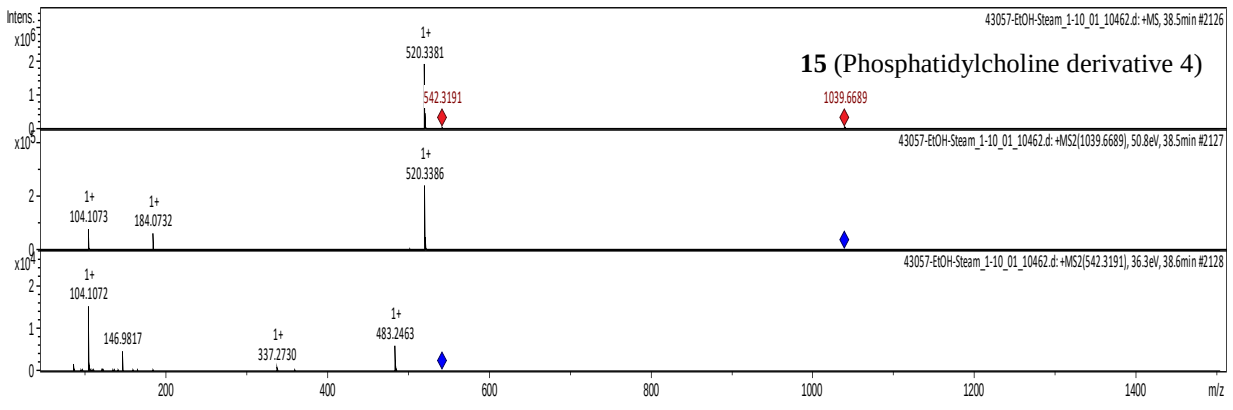
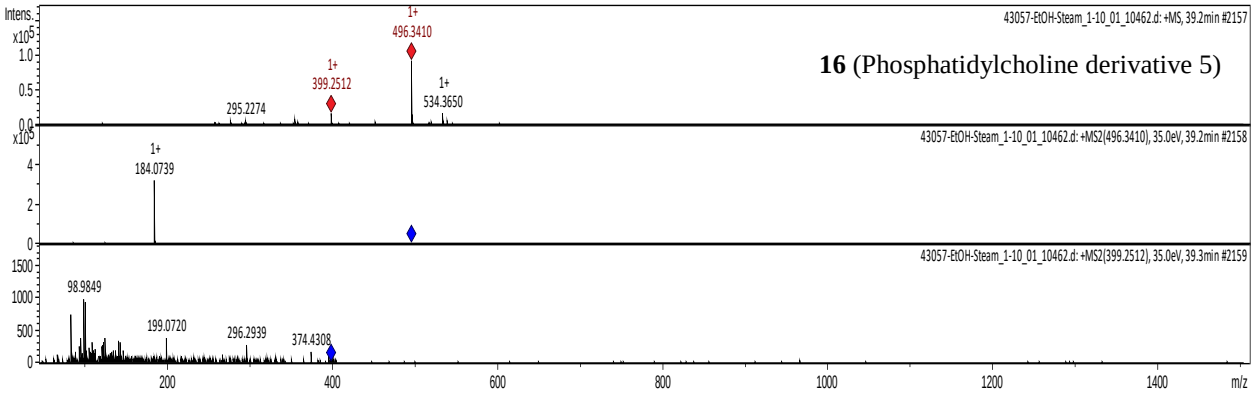
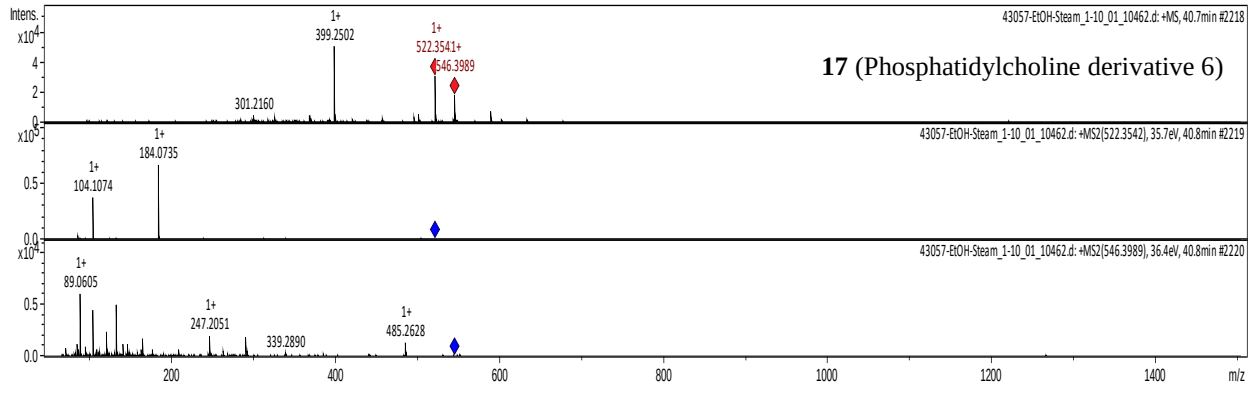


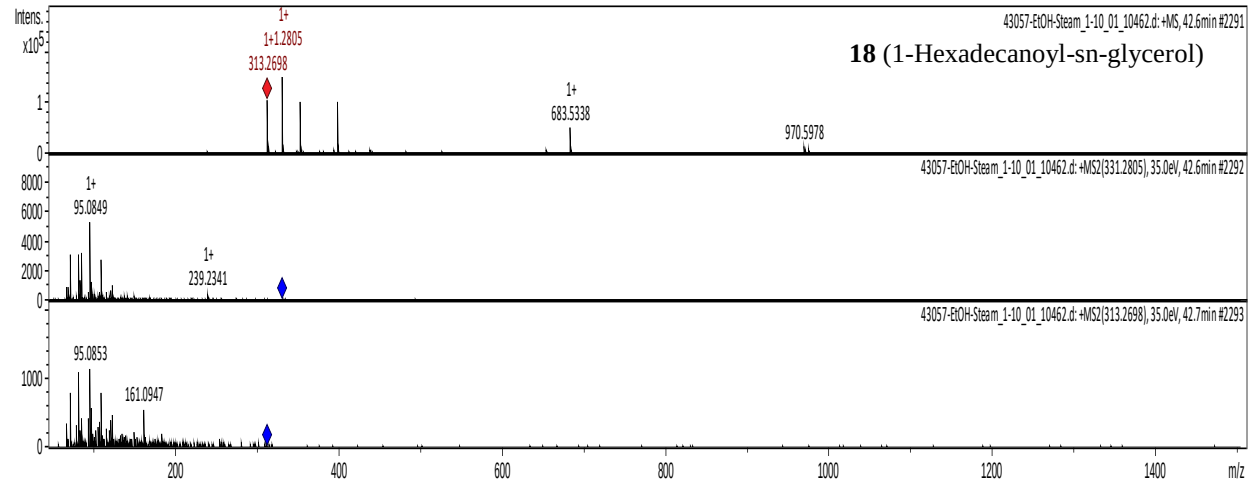
15 (Phosphatidylcholine derivative 4)



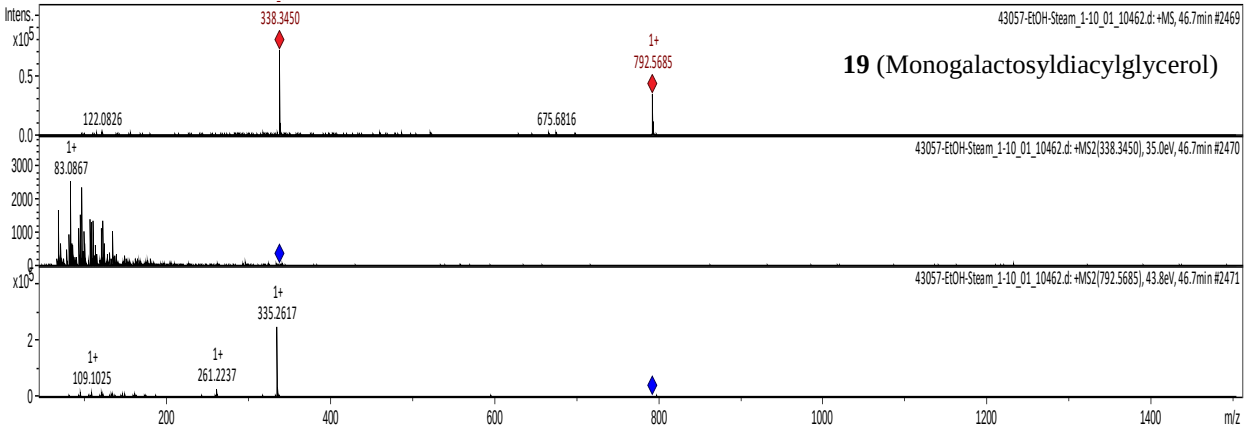
16 (Phosphatidylcholine derivative 5)



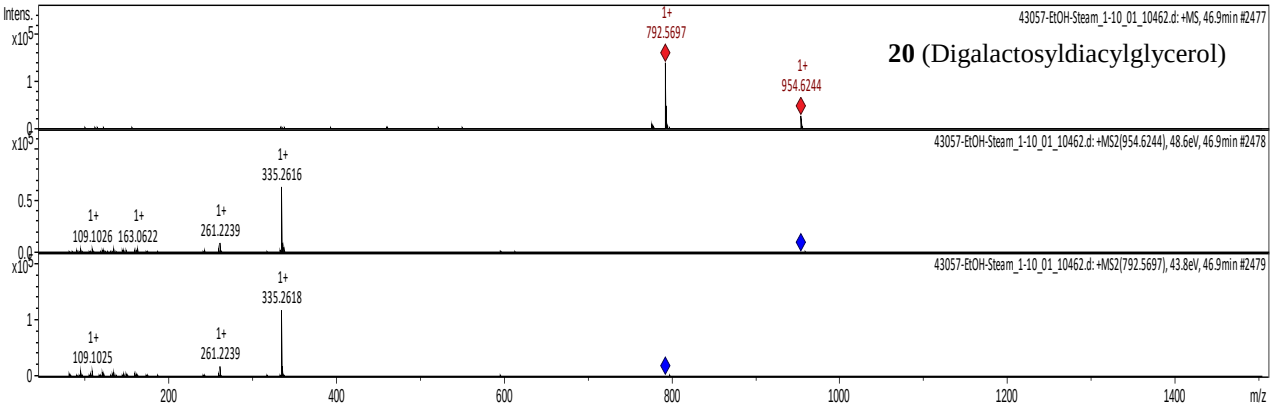
17 (Phosphatidylcholine derivative 6)

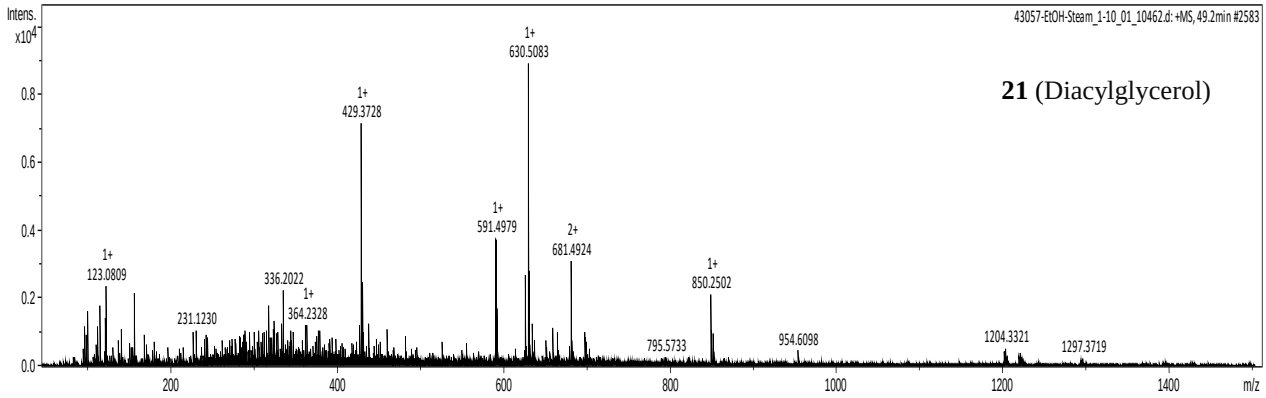


18 (1-Hexadecanoyl-sn-glycerol)



19 (Monogalactosyldiacylglycerol)


Intens.
x10^5
1+
792.5697
1+
954.6244
43057-EtOH-Steam_1-10_01_10462.d: +MS, 46.9min #2477
20 (Digalactosyldiacylglycerol)
43057-EtOH-Steam_1-10_01_10462.d: +MS2(954.6244), 48.6eV, 46.9min #2478
1+
335.2616
1+
109.1026
1+
163.0622
1+
261.2239
43057-EtOH-Steam_1-10_01_10462.d: +MS2(792.5697), 43.8eV, 46.9min #2479
1+
335.2618
1+
109.1025
1+
261.2239
200
400
600
800
1000
1200
1400
m/z


Intens.
x10^4
43057-EtOH-Steam_1-10_01_10462.d: +MS, 49.2min #2583
21 (Diacylglycerol)
1+
630.5083
1+
429.3728
1+
591.4979
2+
681.4924
1+
850.2502
1+
123.0809
336.2022
1+
364.2328
231.1230
795.5733
954.6098
1204.3321
1297.3719
200
400
600
800
1000
1200
1400
m/z