Comparative study of *in-vitro* antioxidant, anti-acetylcholinesterase, anti-xanthine oxidase, anti-5-lipoxygenase, anti-α-amylase and cytotoxic activity of *Acacia* species (Mimosaceae)

Amira Jelassi, Mayssa Ben Mustapha, Mansour Znati, Hichem Ben Jannet,[a,*]

Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity (LR11ES39), Team: Medicinal Chemistry and Natural Products, Faculty of Science of Monastir, University of Monastir, Avenue of Environment, 5019 Monastir, Tunisia

Abstract

The present study aimed the investigation of the acetylcholinesterase, xanthine oxidase (XOD), 5-lipoxygenase, α-amylase, antioxidant and cytotoxic activities of various organic extracts from seeds and pods of two Tunisian species of *Acacia*, *A. cyclops* and *A. mollissima* (Mimosaceae). At concentrations of 50 µg/mL, EtOAc extract of seeds from *A. mollissima* was able to strongly inhibit xanthine oxidase (XOD) with 98.40%. Moreover, EtOAc and n-BuOH extracts from pods of *A. mollissima* showed the highest inhibitor activity against 5-lipoxygenase with percentages of 79.60 and 64.86% inhibition, respectively. However, most of the tested extracts were not good acetylcholinesterase inhibitors. n-BuOH extracts of pods displayed the highest antioxidant activity (IC_{50}= 36.03 to 45.90 µg/mL) for the DPPH assay and confirmed with the ABTS test. The eight extracts of *Acacia* species were tested for their cytotoxic activity *in vitro* using MCF-7, HCT-116, IGROV-1 and OVCAR-3 cell lines. The most active extracts were those of *A. cyclops* against IGROV-1 ranging from 12.9 to 16.1 µg/mL.

Keywords: *Acacia* species, extracts, xanthine oxidase, 5-lipoxygenase, α-amylase, antioxidant, cytotoxic

*Corresponding author: hichem.bjannet@gmail.com

1. Introduction

Medicinal plants have been used since the earliest civilization and its interest has been spread from one population to another, orally or in writing. These natural products have been used in traditional medicine as principal source to fight against various ailments (Berroukche et al., 2020; Ounaissia et al., 2020; Khatiwada et al., 2021, Zhouri et al., 2022). Researchers found 5000 year old clay tablets from the Sumerian civilization which describes the use of plants in the preparation of drugs and food (Awuchi et al., 2019). The use of medicinal plants was widespread in several regions of the world in the fields of pre and postnatal care (Sonitha et al., 2021). Traditional medicinal knowledge on the use of plants by indigenous peoples throughout the world was transmitted either orally or in written form from one generation to the next (Swargiary et al., 2019). By increasingly using the recipes transmitted from our ancestors, the use of medicinal plants has become increasingly sought in the world because of its ecofriendliness, less side effects and affordable cost (Chaudhari, et al., 2020). *Acacia* (Mimosaceae) has a wide range of ecological amplitudes and is distributed in many regions all over the world. The genus includes more than 1350 species (Zheleva-Dimitrova et al., 2021). In spite of the huge number of *Acacia* species, there has been little research on their phytochemistry (Abdel-Farid et al., 2014). The main use of *Acacia* species was as a fodder source but they were excessively used in traditional medicinal for the treatment of many diseases such as diarrhea, urinary tract infections, headaches, sore throat, tuberculosis and bronchial asthma, also useful in high blood pressure, hypoglycemia, inflammation, dysentery and leprosy (Subhan et al. 2018; Mezni et al., 2021). For example, *Acacia nilotica* was noted among the species of *Acacia* which are known as a multipurpose medicinal and pharmaceutical plant. In traditional medicine, *A. nilotica* was used for the treatment of many diseases including tuberculosis, pneumonia, gonorrhea and small pox (Jame, 2018). It also showed a strong antimicrobial activity against both bacteria and fungi (Kumari et al., 2019). *Acacia salicina* was frequently used in such diverse applications as the treatment of inflammatory diseases, as a febrifuge, to treat cancer and to promote human fertility (Mezni et al., 2021). Our investigation involved the screening of extracts from seeds and pods of two Tunisian species of *Acacia*, *A. cyclops* and *A. mollissima* for their acetylcholinesterase, xanthine oxidase, 5-lipoxygenase, α-amylase, antioxidant and cytotoxic inhibitor activities. To the best of our knowledge, these biological activities of the selected medicinal plants grown in Tunisia have not been reported.

2. Materials and methods

2.1. Chemical Reagents and Solvents

Solvents: acetone (99,8%), ethyl acetate (99,5%), n-butanol (99%), DMSO (99.9%), sodium phosphate monobasic (NaH_2PO_4) (99%), gentamycin, nordihydroguaiaretic acid (95%), dinitro salicylic acid (98%), L-glutamine (99%), MTT solution, doxorubicin, cisplatin, Tween and acarbose (95%) were obtained from fisher scientific (part of Thermo Fisher Scientific, Illkirch, France). Sodium phosphate dibasic (Na_2HPO_4) (≥99.0%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), butylated hydroxytoluene (BHT) (≥99%), linoleic acid, galantamine, 5,5′-dithiobis-2-nitrobenzoic acid (99%), α-amylase, acetylcholinesterase, xanthine oxidase, allopurinol and 5-lipoxygenase (≥95%) were purchased from Sigma (St. Louis, MO, USA).

2.2. Plant material

Seeds and pods of two species of *Acacia*, *A. cyclops* and *A. mollissima* were collected from two different regions in Tunisia characterized by different types of climate (*A. cyclops* from Beja: a semi-arid climate, and *A. mollissima* from Kelibia). The studied seeds and pods were picked up in December 2010.

2.3. Organic crude extract preparation

Seeds and pods of the studied species were dried, ground and subjected to maceration in acetone-water (1:1) at a temperature of 25°C. The aqueous solutions, resulting from the evaporation of acetone under vacuum, were subjected to successive extractions with organic solvents with increasing polarity; ethyl acetate (EtOAc) and n-butanol (n-BuOH) to finally give the corresponding extracts (Table 1).

2.4. Biological evaluation

2.4.1. Antioxidant activity

2.4.1.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was measured from the bleaching of purple colored ethanol solution of $DPPH^{\cdot}$ according to the protocol described by

Jlizi et al. (2018). In Brief, 0.5 mL of each extract was mixed with 0.5 mL of DPPH· ethanol solution. After 30 min incubation in the darkness at 25°C, the decrease in absorbance was read at 520 nm using microtitre plates reader. A mixture of 0.5 mL of DPPH· solution and 0.5 mL of ethanol was used as a blank. Butylatedhydroxytoluene (BHT) was used as Standard. The calculated IC_{50} values denoted the concentration required to scavenge 50% of DPPH radicals. The results were expressed in inhibition percentage versus samples concentrations (µg/mL) at 30 min. All the measurements were carried out in triplicate.

2.4.1.2. ABTS radical scavenging activity

The radical scavenging capacity of the samples for the ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical cation was performed according to the protocol described by Jlizi et al. (2018) with slight modifications. A mixture of ABTS (7mM) at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) and potassium persulfate (2.5 mM) was prepared and kept in the dark at room temperature for 16 h. Afterwards, The mixture was diluted with ethanol until it reached an absorbance value of 0.70 ± 0.02 at 734 nm using spectrophotometer. For each sample, diluted solution (100 µL) was allowed to react with the fresh ABTS solution (900 µL), and then the absorbance was read 6 min after the initial mixing. Butylatedhydroxytoluene (BHT) was used as a positive standard. The capacity of free radical scavenging was expressed as IC_{50} (mg/mL) value, which represents the concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

2.4.2. Anti-acethylcholinesterase activity

The Anti-acethylcholinesterase activity of Acacia extracts was determined according to the protocol slightly modified by Filali et al. (2016). 25 µL of each sample, 50 µL of 0.1 M sodium phosphate buffer (pH 8.0) and 25 µL of AChE solution and 125 µL of 5,5′-dithiobis-2-nitrobenzoic acid were mixed and incubated for 15 min at 25°C using a 96-well microplate. All samples were dissolved in the DMSO then diluted in the buffer and the DMSO does not exceed 1% in the mixture. Afterwards, 25 µL of acetylthiocholine iodide solution were added to the initial mixture and the final mixture was incubated at 25°C for 15 min, then the absorbance was

measured at 412 nm. Control contained all components except the tested extracts. Galantamine was used as positive control.

2.4.3. Xanthine oxidase enzyme inhibitory activity (XOD)

To test the inhibitory potency of the enzyme xanthine oxidase by the *Acacia* extracts, we based on the protocol mentioned by Rahamni et al. (2019). A solution of 50 µL of each diluted plant extract, 60 µL of 0.1 mM phosphate buffer (pH = 7.5) and 30 µL of enzyme (xanthine oxidase from bovine milk) solution (0.1 u/ml) prepared from phosphate buffer (pH = 7.5) were prepared before use. The mixture was added in a 96-well microplate and incubated at 25°C for 15 min, then 60 µL of substrate solution (150 mM xanthine in the same buffer). The assay mixture was incubated at 25°C for 30 min and the absorbance was determined at 290 nm. A blank was prepared in the same manner. Result is expressed as the percentage inhibition of xanthine oxidase in the above system, calculated as (1-B/A) × 100, where A and B are the activities of the enzyme without and with test material. Allopurinol was used as positive control.

2.4.4. Anti 5-lipoxygenase activity

To determine the 5-lipoxygenase enzyme inhibitory activity, we used the method described by Aissa et al. (2019). The standard assay mixture contained 12.5 µL of each sample dissolved in DMSO, 50 µL of linoleic acid (0.003 g/10 mL) and made up of 1 mL with 0.1 m phosphate buffer with Tween 0.005 %. The reaction was initiated with the addition of 1.5 µL of 5-lipoxygenase from soybean (0.054 g/mL). The increase in absorbance at 234 nm was recorded for 5 min on a Shimadzu 160-UV spectrophotometer. Nordihydroguaiaretic acid (NDGA) was used as positive control. The percentage inhibition of enzyme activity was calculated by comparison with the negative control: $\%=[(A_0-A_1)/A0]\times100$, where A_0 was the absorbance of the control without extract and A1 was the absorbance of the sample. Tests were carried out in triplicate. IC_{50} was obtained plotting the inhibition percentage against sample concentrations.

2.4.5. α-Amylase inhibitiory activity

The inhibitory effect of the enzyme α-amylase by seeds and pods extracts of the two species of *Acacia* was measured according to the protocol suggested by Hajlaoui et al. (2021) with some alterations. Briefly, 500 µL of each test sample was incubated with 500 µL of α-amylase

solution (0.5 mg/mL in 0.02 M sodium phosphate buffer; pH 6.9 with 0.006 M NaCl). Then, 500 µL of starch solution (1%) in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube after pre-incubation at 25°C for 10 min,. The resulting mixtures were then incubated at room temperature (10 min), and the reaction was stopped using 1 mL of DNS (Dinitro salicylic acid) reagent. At this time, the test tubes were placed in a water bath (100°C) for 5 min and cooled until room temperature was attained. The mixture was then diluted with 10 mL of deionized water, and the absorbance was determined at 540 nm. The readings were compared with the control, containing buffer instead of test extracts. Acarbose was used as a positive control. The inhibition of α-amylase was calculated using the following equation:

$$\% \text{ inhibition of } \alpha\text{−Amylase} = (Abs_{control} - Abs_{sample})/(Abs_{control}) \times 100$$

Where $Abs_{control}$ corresponds to the absorbance of the solution containing only α-amylase and the buffer instead of the compound, and Abs_{sample} corresponds to the absorbance of the solution in the presence of both tested compound and α-amylase. Compound concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against the concentrations of the tested compound. The tests were carried out in triplicate.

2.4.6. Cytotoxic activity

The assays of cytotoxic activity of Acacia extracts were assessed against three types of cells, a human breast cancer cells (MCF-7), human colon cancer cells (HCT-116) and human ovarian cancer (IGROV-1 and OVCAR-3) as described by Filali et al. (2016). Each extract was added to a medium containing 1×10^6 cells/mL, L-glutamine (2 mM) and gentamycin (50 µg/mL), and kept at 37°C in a fully humidified atmosphere. After 18 h of incubation at 37°C in 5% CO_2 incubator, the tubes were centrifuged at 8000g for 10 min. The supernatant was decanted, and the pellets were taken and washed with 20 mM of phosphate-buffered saline solution. Each pellet was dissolved in 100 µL (2 mg/mL) MTT solution in a tube, incubated for 4 h at 22°C and centrifuged for 10 min at 8000g. All the pellets were dissolved in 500 µL DMSO and measured at 500 nm using a spectrophotometer. Doxorubicin (HCT-116 and MCF-7) and Cisplatin (IGROV-1 and OVCAR-3) were used as positive control.

3. Results and Discussion

3.1. Chemistry

Organic extracts obtained from seeds and pods of *Acacia* species presented different yields according to the organ and the solvent (Table 1). *A. cyclops* was the species with the highest yields and especially for butanol extracts (7% for pods and 2.51% for seeds). The most remarkable yield was that of the butanol extract of pods from *A. mollissima* (5.20%).

Table 1. Yield, in percent of dry matter, of organic extracts of the different parts of *Acacia cyclops* and *A. mollissima*

Plant part	Solvent extract	Plant	% Yield
Seeds	EtOAc	*A. cyclops*	0.69
		A. mollissima	0.27
	BuOH	*A. cyclops*	2.51
		A. mollissima	0.71
Pods	EtOAc	*A. cyclops*	0.91
		A. mollissima	0.83
	BuOH	*A. cyclops*	7.00
		A. mollissima	5.20

The phytochemical analysis of organic extracts of seeds and pods of *A. cyclops* and *A. mollissima* has been reported in 2016 (Jelassi et al., 2016). This investigation was concerned with the determination of flavonoid, phenol and condensed tannins contents.

The total flavonoid contents in these extracts were closely ranged (4.20–24.13 mg rutin equivalents/100 g DW), n-butanol extracts of *Acacia* pods were the richest ones in phenol (11.78 to 426.36 mg gallic acid equivalents/ 100 g DW) and condensed tannin (12.55 to 2518.60 mg cyanidin chloride equivalents /100 g DW) contents. These results conclude that *Acacia* extracts are rich in phenolic and tannins contents and these findings encouraged us to study their biological activities.

3.2. Biology

3.2.1. Antioxidant activity

Oxidants have long been known for their health benefits because they have the ability to prevent several diseases such as cancer, and cardiovascular diseases (Ali et al., 2020).

Therefore, the present study aimed to investigate the antioxidant capacities of *Acacia* extracts by DPPH and ABTS assays. Results are shown in Figure 1. The DPPH radical scavenging capacity of extracts varied from IC_{50} = 36.03 to IC_{50} = 489.29 µg/mL. n-BuOH extracts of pods showed the lowest IC_{50} (ranging from 36.03 to 45.90 µg/mL) indicating its highest antioxidant activity. Compared to BHT reference (17.50 ± 1.05 µg/mL), pods of *A. cyclops* and *A. mollissima* have good potential and can be considered as promising antioxidants.

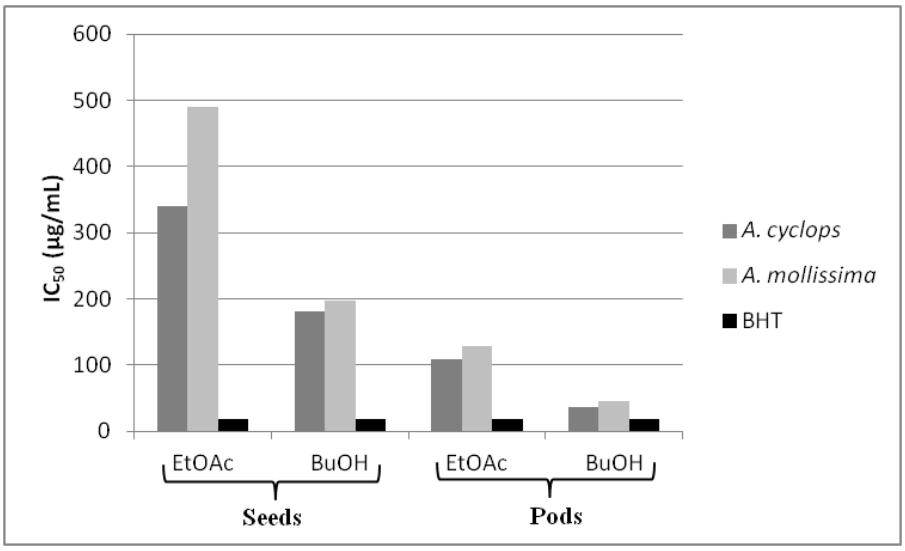
Besides, EtOAc extracts of pods exhibited a significant activity with IC_{50} varied between 109.12 and 128.94 µg/mL. EtOAc and n-BuOH extracts of seeds have imparted a moderate antioxidant effect (179.90< IC_{50}< 489.29 µg/mL)

The ABTS assay confirmed the DPPH assay results. Among the eight extracts, n-BuOH extracts of pods were the most potent according to ABTS radical scavenging activity with IC_{50} value ranging from 77.30 to 86.90 µg/mL, and followed by EtOAc extracts of the same vegetative part with IC_{50} value aligned between 186.30 and 219.63 µg/mL.

Other extracts of seeds from the two species of *Acacia* revealed a weak activity (290.71<IC_{50}<695.82 µg/mL).

The antioxidant activity of *Acacia* extracts were checked as it was expected that the extracts having good antioxidant activities would be rich in phenolic compounds. As the n-BuOH extracts of pods exhibited a maximum antioxidant activity, so the total phenol contents were also observed to be very high. The difference observed in the IC_{50} mentioned above could be explained in large part by a difference in the contents of these phenolic compounds and also by a difference in the structure of these compounds responsible for this antiradical activity. By comparison with other works, a study from Australia performed by Chong et al. (2019) found a significant radical scavenging capacity at 10 mg/mL with 27.8% in wattle seeds extracts of *Acacia cyclops*. As well as that, the antioxidant activity of other *Acacia* species attracted several researchers to discover it. For example, Kumar et al. (2018) noted that methanol extract of *Acacia catechu* exhibited a moderate DPPH radical and ABTS radical scavenging activities with IC_{50} values of 101.74 and 140.41 µg/mL, respectively. In addition, Sulaiman et al. (2013) noted that *Acacia catechu*, *Acacia leucophloea* and *Acacia nilotica* possessed a significant DPPH radical scavenging capacity with IC_{50} values of 13.16, 18.38 and 12.52 µg/mL.

a)



b)

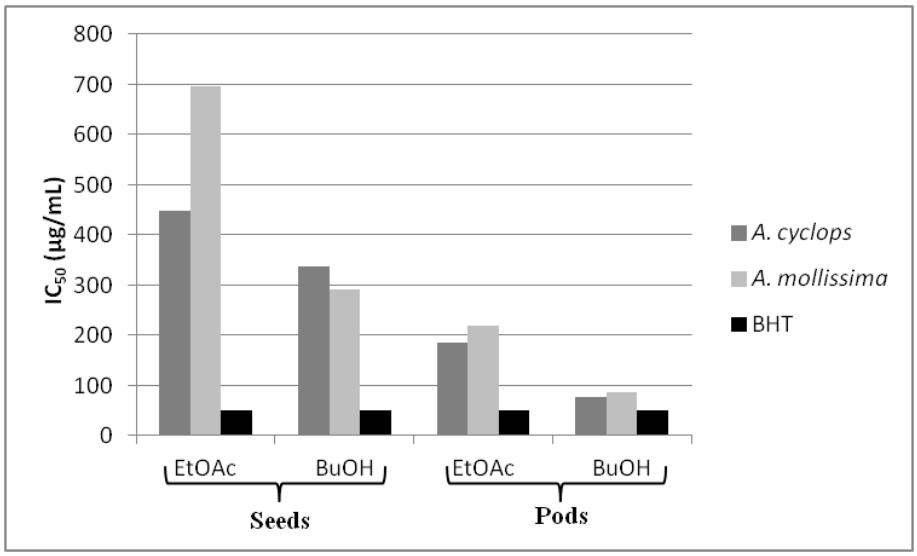


Figure 1. Antioxidant activity ($DPPH^{\cdot}$ (a) and $ABTS^{+\cdot}$ (b)) of organic extracts from *A. cyclops* and *A. mollissima* plant parts.

3.2.2. Acetylcholinesterase (AChE) enzyme inhibitory activity

Alzheimer's disease is a neurodegenerative disorder that is defined by progressive loss of memory and other cognitive functions (Altiné-Samey et al., 2021). The current solutions aim only to decrease the progression of this disease by using anti-cholinesterase inhibitors (Vecchio et al., 2021). Current research aim to have natural inhibitors from medicinal plants to enhance cognitive functions and to endure other symptoms associated with Alzheimer's disease (Ahmed et al., 2021). In this subject the anti-acetylcholinesterase activity of *Acacia* extracts was tested

(Table 2). Results showed that all extracts were considered not active at a concentration of 50 µg/mL. Inhibition percentages ranged from 3.35 to 22.77%.

To the best of our knowledge, no study of the anti-acetylcholinesterase activity of seeds and pods extracts from these two species of *Acacia* was reported in the literature. However, diverse studies investigated the anti-acetylcholinesterase activity of other species of *Acacia* and same results were reported. Ethanolic extracts of *A. catechu*, *A. leucophloea* and *A. nilotica* originating from India were found to be inactive at a concentration of 1 mg/mL (Sulaiman et al., 2013). In another research, it was found that ethanol extract of *A. catechu* seeds inhibits acetylcholinesterase with an IC_{50} value of 204.38 µg/mL (Thangavelu and Ramasamy, 2015).

Table 2. Acetylcholinesterase, xanthine oxidase, 5-lipoxygenase and α-amylase inhibitory activities of organic extracts from *Acacia cyclops* and *A. mollissima* plant parts expressed in percentage of inhibition at 50 µg/mL.

Plant part	Solvent extract	Plant	AChE Activity	XOD activity	5-LOX activity	α-amylase Activity
Seeds	EtOAc	*A. cyclops*	7.13 ± 1.01*	32.47 ± 0.77	6.06 ± 0.20	49.15 ± 1.41
		A. mollissima	6.14 ± 0.99	98.40 ± 0.86 (9.22 ± 0.97)**	4.82 ± 0.09	50.15 ± 0.29
	BuOH	*A. cyclops*	21.48 ± 0.84	51.72 ± 2.92 (49.05 ± 02.14)**	1.56 ± 0.17	67.16 ± 0.89 (38.18 ± 1.71)**
		A. mollissima	22.41 ± 0.8	42.11 ± 0.60	5.37 ± 0.72	64.49 ± 1.71 (42.35 ± 1.00)**
Pods	EtOAc	*A. cyclops*	9.58 ± 0.77	22.80 ± 0.87	11.72 ± 0.79	35.82 ± 1.01
		A. mollissima	22.77 ± 0.70	47.26 ± 1.95	79.60 ± 1.72 (16.08 ± 1.11)**	35.21 ± 1.22
	BuOH	*A. cyclops*	3.35 ± 0.08	45.53 ± 1.27	8.90 ± 0.17	50.23 ± 1.62
		A. mollissima	14.73 ± 0.95	43.51 ± 0.84	64.86 ± 1.99 (23.45 ± 1.55)**	68.37 ± 1.69 (35.04 ± 1.22)**

Galanthamine	1.16 ± 0.04**			
Allopurinol		1.13 ± 0.04**		
NDGA			63.2 ± 1.24**	
Acarbose				0.51 ± 0.024**

*:percent inhibition. **: IC_{50} (µg/mL).

3.2.3. Xanthine oxidase enzyme inhibitory activity (XOD)

The XOD inhibitory effects of the EtOAc and n-BuOH extracts from *A. cyclops* and *A. mollissima* were evaluated at a concentration of 50 µg/mL and compared with literature.

A remarkable difference in inhibition percentages was noticed whatever depending on the nature of the solvent extract or the plant material (Table 2).

As shown in table 2, EtOAc extract of seeds from *A. mollissima* exhibited a strong inhibitory activity against the XOD-induced superoxide formation with percentage inhibition of 98.40%. However, the n-BuOH extract of the same organ from the same species has displayed a moderate inhibition with a percentage of 42.11%. The relatively high activity in EtOAc extract of *A. mollissima* seeds could be attributed to the nature of the compounds present in this extract. Therefore *A. mollissima* may contain bioactive substances useful in the treatment of gout or other XOD-induced diseases, justifying the popular use of these species in traditional medicines. Moreover, the recorded results showed that n-BuOH extract of seeds from *A. cyclops* was able to inhibit XOD enzyme with 51.72% at 50 µg/mL. On the other side, its EtOAc extract moderately inhibited this enzyme (32.47%). n-BuOH extracts of pods from *Acacia* species showed a modest inhibition and similar percentages of inhibition with 43.51 and 45.53 % for *A. mollissima* and *A. cyclops*, respectively.

EtOAc extracts of pods from *Acacia mollissima* has a moderate inhibition percentage 47.26%, in contrast for EtOAc extracts of pods from *Acacia cylops* which was not active (22.80 %).

In fact, Sweeney et al. (2001) reported that EtOH:H_2O (4:1) extract of leaves and branches from *Acacia melanoxylon* exhibited an inhibition percentage at 100 µg/mL of 53.69%, IC_{50}= 61.9 µg/mL. Heartwood, bark, twig, flower, and leaf ethanolic crude extracts of *Acacia confusa* were tested also at 100 µg/mL by Tung and Chang, (2010), the XOD inhibition of *A. confusa* decreased in the following order: heartwood extract (80%)>bark extract (59%)>flower extract (46%) = leaf extract (45%) > twig extract (39%).

Results indicate that extracts of some species of *Acacia* have a good performance of inhibiting superoxide free radicals produced by xanthine oxidase.

3.2.4. 5-Lipoxygenase enzyme inhibitory activity

Inflammation is a complicated biological response of vascular tissues. Steroidal and non-steroidal drugs are best known for their effectiveness against inflammatory diseases but also for their several undesirable effects. Therefore the researchers were obliged to look for new sources of anti-inflammatory compounds and above all they are aimed at the anti-inflammatory agents of natural sources (Chel-Guerrero et al., 2022).

The present study was focused to evaluate lipoxygenase inhibitory activity of crude seeds and pods extracts of *Acacia* species. Results of 5-lipoxygenase enzyme inhibitory activity of the tested extracts are shown in Table 2. EtOAc and n-BuOH seed extracts of the two species of *Acacia* showed no activity against the 5-lipoxygenase enzyme.

Also for the pods extracts, no activity was noticed excluding those of *A. mollissima*. In fact, at 50 µg/mL EtOAc and n-BuOH extracts from pods of *A. mollissima* showed the highest activity with percentages of inhibition of 79.60 and 64.86%, respectively.

Few studies have been interested in effect of *Acacia* species in 5-lipoxygenase activity but we can quote some example of their effects in inhibition of other enzymes like COX-1 and COX-2 (Cyclooxygenase assays). Ethanolic extracts of leaf and bark from different species of Acacia (*A. farnesiana*, *A. longifolia* and *A. tortilis*) were investigated for inhibition of COX-1 and COX-2 enzymes. Most extracts from the three species exhibited strong inhibition of both COX-1 and COX-2 (Gabr et al., 2018).

3.2.5. α-Amylase enzyme inhibitory activity

Diabetes is a chronic metabolic disease defined by hyperglycemia provoked by dysfunction in carbohydrate, protein and fat metabolism due to the insufficiency of insulin produced by the body, or the wrong response of cells to the insulin produced (Sun et al., 2021).

Human α-amylase is one of the major secretory products of the salivary glands and pancreas (Date et al., 2020).

The inhibitory activity of *Acacia* species extracts against α-amylase is shown in Table 2. Extracts from *A. cyclops* and *A. mollissima* (seeds and pods) were classified as moderately active. The range of percentages of inhibition values was from 35.21 to 68.73% at 50 µg/mL.

EtOAc extracts of pods from *A. cyclops* and *A. mollissima* showed the weakest activity inhibitory with percentage of inhibition of 35.82 and 35.21%, respectively.

Extracts of seeds from *A. cyclops* and *A. mollissima* have approximately the same activity inhibitory against l'α-amylase enzyme with percentages of inhibition of 49.15 and 50.15% for the EtOAc extracts and 67.16 and 64.49% for the n-BuOH ones.

Buttner el al. (2021) evaluated the inhibitory effect on α-amylase of pods from *A. cyclops* originating from Australia. The IC_{50} values of ethanolic and aqueous extracts were 52.78 and 52.24 µg/mL, respectively. In the case of *Acacia catechu*, Aryal et al. (2021) proved that ethanol extract of Bark has a significant α-amylase activity with an IC_{50} value of 67.8 24 µg/mL.

3.2.6. Cytotoxic activity

The results of the cytotoxic test on *Acacia* extracts using MCF-7, HCT-116, OVCAR-3 and IGROV-1 cell lines are given in Table 3.

By comparing the IC_{50} of the various extracts tested towards different cellular lines, it is shown that the IGROV-1 line is the most sensitive to the various *Acacia* extracts tested. As determined by the MTT essay, the most active extracts were those from *A. cyclops* against IGROV-1 decreased in the following order: EtOAc extract of seeds (16.1 µg/mL) > n-BuOH of seeds (15.5 µg/mL) >EtOAc extract of pods (14.1 µg/mL) > n-BuOH of pods (12.9 µg/mL).

The toxic effect of n-BuOH of seeds from *A. mollissima* against the same cell lines showed a moderate activity with an IC_{50}= 39 µg/mL, however the EtOAc extract of the same part did not present any activity. Despite the fact that extracts from *A. cyclops* were active against the ovarian cancer cell lines IGROV-1, they present an IC_{50}>50 µg/mL against OVCAR-3 so recorded not active barring the n-BuOH of pods (IC_{50}= 40 µg/mL). n-BuOH extracts of pods from *A. cyclops* and *A. mollissima* showed a moderate activity with an $IC_{50,}$ respectively, 46 µg/mL and 41 µg/mL against the cell lines MCF-7. Contrary, the rest of the extracts have an IC_{50}>50 µg/mL.

For the HCT-116 cell lines, only extracts of pods from *A. mollissima* have a moderate toxic effect against this cell line, IC_{50}= 37 µg/mL for EtOAc extract and $IC_{50=}$ 41 µg/mL for the n-BuOH one. The cytotoxic effect of *A. cyclops* pods and seeds extracts on human cell line (Caco-2) was reported by Buttner et al. (2021).

Table 3. Cytotoxicity of organic extracts from *Acacia cyclops* and *A. mollissima* plant parts.

[a]IC_{50} values represent the mean ± standard deviation of three parallel measurement (P<0.05).
Cancer cell lines: IGROV-1 and OVCAR-3: ovarian cancer cell lines, MCF-7 a breast cancer cell line and HCT-116: a human colon cancer cell line
[b]Cisplatin
[c]Doxorubicin

4. Conclusion

For a long time, traditional medicine has been used from one civilization to another and until today it still persists and is subject to a competition with modern medicine.

Despite the lack of standardization in traditional medicine with respect to raw materials, methods of production and in quality control of the finished product, the use of this medicine based on medicinal plants is continuous from one generation to the next and provides the supply of new products considered potential sources in the manufacture of effective and safe drugs. To the best of our knowledge, this is the first study that reports on the acetylcholinesterase, XOD, 5-lipoxygenase and α-amylase inhibitory, antioxidant and cytotoxic activities of two species of *Acacia*, *A. cyclops* and *A. mollissima* grown in Tunisia.

Plant part	Solvent extract	Plant	IC_{50} (µg/mL)[a]			
			IGROV-1	OVCAR-3	MCF-7	HCT-116
Seeds	EtOAc	*A. cyclops*	16.1 ± 2.7	>50	>50	>50
		A. mollissima	>50	>50	>50	>50
	BuOH	*A. cyclops*	15.5 ± 3.1	>50	>50	>50
		A. mollissima	39.0 ± 4.3	>50	>50	>50
Pods	EtOAc	*A. cyclops*	14.1 ± 2.0	>50	>50	>50
		A. mollissima	>50	>50	>50	37.0 ± 3.7
	BuOH	*A. cyclops*	12.9 ± 1.4	40.0 ± 5.0	46.0 ± 7.0	>50
		A. mollissima	>50	48.0 ± 6.1	41.0 ± 7.0	41.0 ± 3.0
Cispl[b]			0.04 ± 0.00	3.53 ± 0.50		
Doxor[c]					0.22 ± 0.03	0.12 ± 0.01

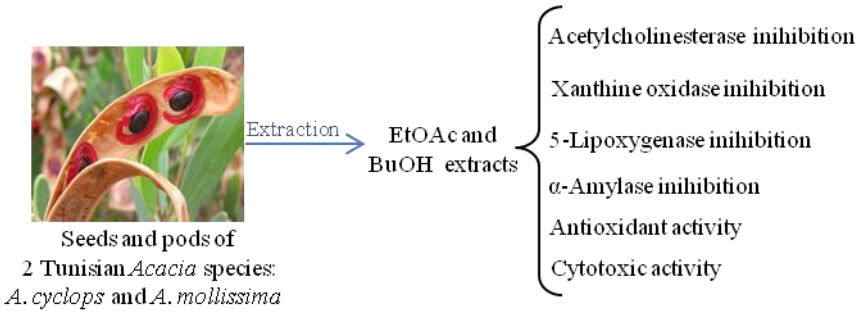
We can conclude that a remarkable difference in inhibition effects was noticed whatever depending on the nature of the solvent extract, the plant material or the species of *Acacia*. It is clear from the results that *Acacia* species are a greatly rich source of bioactive compounds. Nevertheless, it is interesting to continue the fractionation work to isolate the molecules responsible for these activities or to exploit and test other species given the abundance of this genus in the world.

Acknowledgements

The authors are grateful to Prof. Fethia Harzallah-Skhiri, High Institute of Biotechnology of Monastir, Tunisia, for botanical identification, to Nabeul Forest Department (Ministry of Agriculture, Tunisia) for the supply of plant material and to the Ministry of Higher Education and Scientific Research of Tunisia, General Directorate for Scientific Research, for the financial support to our Laboratory.

Author Contributions: Conceptualization and methodology, A.J. and M.B.M.; validation, M.Z and H.B.J., formal analysis; A.J. and M.Z., investigation, A.J. and M.B.M; writing—original draft preparation, A.J. and M.B.M.; writing—review and editing, H.B.J.; visualization, H.B.J.; supervision, H.B.J.; project administration, H.B.J. and M.Z.; All authors have read and agreed to the published version of the manuscript.

Graphical Abstract



References

Abdel-Farid, I. B., Sheded, M. G., Mohamed, E. A., 2014. Metabolomic profiling and antioxidant activity of some Acacia species. Saudi J. Biol. Sci. 21(5), 400-408.

Ahmed, S., Khan, S. T., Zargaham, M. K., Khan, A. U., Khan, S., Hussain, A., Uddin, J., Khan, J., Al-Harrasi, A., 2021. Potential therapeutic natural products against Alzheimer's disease with Reference of Acetylcholinesterase. Biomed. Pharmacother. 139, 111609.

Aissa, I., Nimbarte, V. D., Zardi-Bergaoui, A., Znati, M., Flamini, G., Ascrizzi, R., & Jannet, H. B. (2019). Isocostic acid, a promising bioactive agent from the essential oil of Inula viscosa (L.): Insights from drug likeness properties, molecular docking and SAR analysis. Chem. Biodivers. 16(4), e1800648.

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., Khan, F. H., 2020. Understanding oxidants and antioxidants: Classical team with new players. J. Food biochem. 44(3), e13145.

Altiné-Samey, R., Antier, D., Mavel, S., Dufour-Rainfray, D., Balageas, A. C., Beaufils, E., Emond, P., Foucault-Fruchard, L., Chalon, S., 2021. The contributions of metabolomics in the discovery of new therapeutic targets in Alzheimer's disease. Fundam. Clin. Pharmacol.35(3), 582-594.

Aryal, B., Adhikari, B., Aryal, N., Bhattarai, B. R., Khadayat, K., Parajuli, N., 2021. LC-HRMS profiling and antidiabetic, antioxidant, and antibacterial activities of Acacia catechu (Lf) Willd. BioMed. Res. Int. 2021.

Awuchi, C. G., 2019. Medicinal plants: the medical, food, and nutritional biochemistry and uses. Int. J. Adv. Acad Res. 5(11), 220-241.

Berroukche, A., Halla, N., Terras, M., Ferhat, R.F., 2020. Drugs and herbs in two divergent lines of benign prostatic hyperplasia therapy. AJMAP, 6(1), 92-104.

Buttner, D. H., Reddy, S., Koekemoer, T., van de Venter, M., 2021. An in vitro assessment of the potential antidiabetic activity and cytotoxic effects of ethanolic and aqueous extracts from three invasive Australian acacias. S. Afr. J. Bot. 141, 1-11.

Chaudhari, A. K., Das, S., Singh, B. K., Prasad, J., Dubey, N. K., Dwivedy, A. K. 2020. Herbal medicines as a rational alternative for treatment of human diseases. In Botanical Leads for Drug Discovery. pp. 29-49. Springer, Singapore.

Chel-Guerrero, L. D., Castañeda-Corral, G., López-Castillo, M., Scampicchio, M., Morozova, K., Oney-Montalvo, J. E., Ferrentino, G., Acevedo-Fernandez, J. J., Rodríguez-Buenfil, I. M., 2022. In Vivo Anti-Inflammatory Effect, Antioxidant Activity, and Polyphenolic Content of Extracts from Capsicum chinense By-Products. Molecules 27(4), 1323.

Chong, W. S. C., Dykes, G. A., Coorey, R., 2019. Chemical Composition and Anti-Nutritional Profiling of Wattle (*Acacia cyclops*, *Acacia microbotrya* and *Acacia victoriae*) Seed Originating from Western Australia. Plant Foods Hum. Nutr. 74(4), 518-524.

Date, K., Yamazaki, T., Toyoda, Y., Hoshi, K., Ogawa, H., 2020. α-Amylase expressed in human small intestinal epithelial cells is essential for cell proliferation and differentiation. J. Cell. Biochem. 121(2), 1238-1249.

Filali, I., Belkacem, M. A., Ben Nejma, A., Souchard, J. P., Ben Jannet, H., Bouajila, J., 2016. Synthesis, cytotoxic, anti-lipoxygenase and anti-acetylcholinesterase capacities of novel derivatives from harmine. J. Enzyme Inhib. Med.Chem. 31(sup1), 23-33.

Gabr, S., Nikles, S., Wenzig, E. M. P., Ardjomand-Woelkart, K., Hathout, R. M., El-Ahmady, S., Abdel Motaal, A., Singab, A., Bauer, R., 2018. Characterization and optimization of phenolics extracts from Acacia species in relevance to their anti-inflammatory activity. Biochem. Syst. Ecol. 78, 21-30.

Hajlaoui, A., Laajimi, M., Znati, M., Ben Jannet, H., Romdhane, A. 2021. Novel pyrano-triazolo-pyrimidine derivatives as anti-α-amylase agents: Synthesis, molecular docking investigations and computational analysis. J. Mol. Struct. 1237, 130346.

Jame, R., 2018. Phytochemical and pharmacological uses of acacia nilotica-a review. Int. J.Bioorg. Chem. 1, 15-21.

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

Jlizi, S., Zardi-Bergaoui, A., Znati, M., Flamini, G., Ascrizzi, R., & Jannet, H. B. (2018). Chemical composition and biological evaluation of the resin from Tetraclinis articulata (Vahl.) Masters: A promising source of bioactive secondary metabolites. Ind.Crops Prod. 124, 74-83.

Khatiwada, H., Neupane, A., Thapa, L. B., 2021. Traditional Approach to Cure Shingles Using Medicinal Plants in Eastern Nepal. J. Ayurvedic Herb. Med. 7(2), 66-70.

Kumar, R., Arora, R., Mahajan, J., Mahey, S., Arora, S., 2018. Polyphenols from cutch tree (Acacia catechu Willd.): normalize in vitro oxidative stress and exerts antiproliferative activity. Braz. Arch. Biol. Technol. 61.

Kumari, R., Mishra, R. C., Yadav, A., Yadav, J. P., 2019. Screening of traditionally used medicinal plants for their antimicrobial efficacy against oral pathogens and GC-MS analysis of Acacia nilotica extract.

Mezni, F., Khemiri, H., Khemiri, T., Khaldi, A. 2021. Phenolic content and antifungal activity of extracts from Acacia salicina L. Arab. J. Chem. Environ. Res. 8(01), 150-158.

Ounaissia, K., Nassar, A., Soltani, B., Bennadja, S., Djahoudi, A., 2020. Anti-bacterial activity of methanolic extracts of the aerial parts of I*nula viscosa* l., from Guelma (Algeria). AJMAP. 6(3), 10-19.

Rahmani, R., Beaufort, S., Villarreal-Soto, S. A., Taillandier, P., Bouajila, J., Debouba, M., 2019. Kombucha fermentation of African mustard (*Brassica tournefortii*) leaves: Chemical composition and bioactivity. Food Biosci. 30, 100414.

Sonitha, S., Kumar, S. P., 2021. Traditional practices of postnatal care using medicinal plants in Tirunelveli district, Tamil Nadu, India. J. Med. Plants. 9(5), 59-62.

Subhan, N., Burrows, G. E., Kerr, P. G., Obied, H. K., 2018. Phytochemistry, ethnomedicine, and pharmacology of Acacia. Stud. Nat. Prod. Chem. 57, 247-326.

Sulaiman, C.T., Sadashiva, C.T., Satheesh, G., Goplakrishnan, V.K., Balachandran, I., 2013. Chromatographic studies and in vitro screening for Acetyl Cholinesterase Inhibition and Antioxidant Activity of three *Acacia* Species from South India. Anal. Chem. Lett. 3(2), 111-118.

Sun, C., Liu, Y., Zhan, L., Rayat, G. R., Xiao, J., Jiang, H., Li, X., Chen, K., 2021. Anti-diabetic effects of natural antioxidants from fruits. Trends Food Sci. Technol. 117, 3-14.

Swargiary, A., Roy, M. K., Daimari, M., 2019. Survey and documentation of ethnobotanicals used in the traditional medicines system of tribal communities of Chirang district of Assam against helminthiasis. Biomed. Pharmacol. J. 12(4), 1923-1935.

Sweeney, A.P., Wyllie, S.G., Shalliker, R.A., Markham, J.L., 2001. Xanthine oxidase inhibitory activity of selected Australian native plants. J. Ethnopharmacol. 75, 273-277.

Thangavelu, L., Ramasamy, R., 2015. In vitro acetyl cholinesterase inhibitory assay of Acacia catechu willd ethanolic seed extract. *Pharmacogn. J. 7*(5).

Tung, Y.T., Chang, S.T., 2010. Inhibition of Xanthine Oxidase by *Acacia confusa* extracts and their phytochemicals.J. Agric. Food Chem. 58, 781-786.

Vecchio, I., Sorrentino, L., Paoletti, A., Marra, R., Arbitrio, M., 2021. The State of The Art on Acetylcholinesterase Inhibitors in the Treatment of Alzheimer's Disease. J. Centr. Nerv. Syst. Dis.13, 11795735211029113.

Zheleva-Dimitrova, D., Sinan, K. I., Etienne, O. K., Ak, G., Sharmeen, J. B., Dervisoglu, G., Ozdemir, F.A., Mahomoodally, M.F., Zengin, G., 2021. Comprehensive chemical

characterization and biological evaluation of two *Acacia* species: *A. nilotica* and *A. ataxacantha*. Food Chem. Toxicol. 156, 112446.

Zouhri, A., Bouddine, T., El menyiy, N., El-mernissi, Y., El-Akhal, J., Hajji, L., Amhamdi, H., 2022. *Syzygium aromaticum*: Traditional uses, antioxidant, anti-inflammatory activities and photo-protective properties. AJMAP, 8(3), 43-56.

Arabian Journal of Medicinal and Aromatic Plants,

ISSN 2458-5920, www.ajmap.info