IJBPAS, October, Special Issue, 2023, 12(10): 228-250
ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)
'A Bridge Between Laboratory and Reader'
www.ijbpas.com

PHYTOCHEMICAL STUDY AND EVALUATION OF THE ANTIBACTERIAL, ANTIOXIDANT, CYTOTOXIC AND PHARMACOLOGICAL PROPERTIES OF *Typha australis* Schum & Thonn EXTRACTS

RAVINDRA PATTABHI [1] AND MANNAM SUBBARAO[2*]

1: Research Scholar, Department of Biochemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Andhra Pradesh

2: Professor, Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Andhra Pradesh

[*]**Corresponding Author: Mannam Subbarao: E Mail:** mannamsrao@gmail.com

Received 5th July 2023; Revised 6th Aug. 2023; Accepted 14th Sept. 2023; Available online 15th Oct. 2023

https://doi.org/10.31032/IJBPAS/2023/12.10.1024

ABSTRACT

Medicinal plants have been used for centuries to treat numerous diseases and contain bioactive compounds with potential curative effects. Based on ethnobotanical information, phytochemical and biological studies of medicinal plants have allowed the development of valuable drugs. *Typha australis* Schum & Thonn is a little-studied plant native to India. It is desired to determine the presence of secondary metabolites such as flavonoids and tannins, which are phenolic compounds with antioxidant and bactericidal properties. The objective of the present study is to characterise the phytochemistry, pharmacology, cytotoxic effects, and antibacterial and antioxidant efficacy of *T. australis* leaf extracts. Phytochemical analysis was done using standard procedure colour identification tests, and the antibacterial activity was evaluated using the broth microdilution technique. The level of antioxidant activity was measured using the DPPH free radical test. High-performance liquid chromatography was used to determine the chemicals in the leaf extract. By doing a phytochemical analysis, the flavonoids, phenols, and tannins were found. Antimicrobial activity against *Klebsiella pneumoniae* (8mg/mL), *Candida dubliniensis* (1044 mg/mL) and *Candida metapsilosis* (65 mg/mL), and *Staphylococcus epidermidis* (4mg/mL) can be associated to the chemical compounds found in the ethanolic extract of *T. australis* leaves. This shows that there may be

a way to treat highly resistant yeasts and bacteria. The antioxidant activity of ethanol extract was IC_{50} was 18.67g/mL The findings suggested that the *T. australis* extract does not interfere with cell division because none of the tested doses proved dangerous. This research demonstrated the antibacterial and antioxidant properties of *T. australis* ethanolic extract and its non-toxicity, suggesting the possibility of novel antibiotics for treating infections.

Keywords: *T. australis,* leaf extracts, Phytochemistry, Pharmacology, HPLC, Antimicrobial activity, Antioxidant activity

1. INTRODUCTION

Plants have been used for medicinal purposes by humans since prehistoric times, and they continue to play an essential role in the health of most of the world's population, despite advances in modern medicine **[1]**. It is known that plant species are sources of secondary metabolites with different biological activities, and currently, the interest in antioxidant properties is also frequently reported in many studies. Methodologies ranging from preliminary phytochemical analysis to bio-guided systematic chemical studies can be used to determine medicinal plants' chemical composition and identify their biologically active constituents **[2]**. The goal of a preliminary phytochemical survey is to determine whether or not a plant species contains the following metabolite groups: alkaloids, anthraquinones and naphthoquinones, steroids and triterpenes, flavonoids, tannins, saponins, coumarins, terpene lactones, and cardiotonic. Because each of these compounds is associated with specific biological activities, the preliminary phytochemical study results can guide subsequent investigations to determine the biological activity of the species in question and the active ingredients involved.

Antioxidant substances are essential in capturing different reactive species, including free radicals, which we constantly produce during our metabolism. Such species may be involved in cellular ageing and the development of several chronic-degenerative diseases, atheroma formation, emphysema, and asthma, as well as they can lead to DNA damage and contribute to the development of cancer **[3]**. Typha is a genus with a vast worldwide distribution of approximately 12 species. Its distribution in India and other parts of the world is fundamentally conditioned by temperature, with *T. angustifolia* and *T. latifolia* corresponding to cold, temperate, and subtropical regions, and *Typha australis* Schum & Thonn (synonym:*Typha domingensis*) to the tropical region **[4]**. However, in southeastern India, the three species reach sympatric the latter, *Typha australis* Schum & Thonn, is generally well represented throughout the tropics. *Typha*

australis Schum & Thonn (Figure 1) is a monocotyledon belonging to the Typhaceae Family **[5]**. One of the current concerns is the rapid and widespread expansion of an aquatic weed **[6]**. Its common name is "Totora". It is a perennial, rhizomatous herb, 1 to 2.5 m tall. It has leaves with linear, ribbon-shaped blades, 40-120 cm long by 0.5 to 2 cm wide, with a flat upper face and a slightly convex lower face. It is a species with a wide worldwide distribution. *Typha australis* grows well along the banks of rivers and canals and in temporary ponds, backwaters, channels, rice fields, and irrigation canals **[7]**. Traditional medicine has traditionally relied on Typha species to treat neurological disorders and skin malignancies. Traditional Turkish medicine uses the female flowers of the Typha species topically to cure wounds, burns, and other types of external bleeding. The roots can be utilised as a cytotoxin, astringent, or diuretic **[8]**, anti-inflammatory, antioxidant, and antioxidant **[9]**. Pollens have been connected to various actions by pharmacological study, including cyclic adenosine monophosphate (cAMP) induction, cholesterol reduction, immune system suppression, and the prevention of blood clot formation. All known data in the literature demonstrate the significant medicinal potential of *Typha australis* Schum. & Thonn **[10]**. Very few studies available in the literature that *Typha australis* leaves have the potential to produce bioactive compounds detected the presence of phenols, tannins, saponins and coumarins in ethanolic extracts of pollen grains extract. The use of *T. australis* leaves as an herbal medicine with antimicrobial properties has been described in some parts of the world, such as Turkey and India, where its people use this plant to treat infectious processes, where its phenolic chemical compounds have antioxidant activity, and new evidence suggests anti-inflammatory and antimicrobial activities. Thus, due to the interest in research regarding the bioactivity of products of plant origin and the use of *T. australis* by the population in treating different diseases, it becomes interesting to evaluate the biological activities, including antimicrobial and antioxidant, of this plant species.

2. MATERIAL AND METHODS

2.1 Collection and Identification of plant material: This is experimental and quantitative research. Plant material (*Typha australis* leaves) was collected from widespread cultivation in agricultural fields of Guntur, Andhra Pradesh, India, collected in February 2018. Professor TN Mary confirmed the botanical identity of the plant, and a specimen (2211/18) was deposited in the Herbarium of the Taxonomy laboratory of Acharya Nagarjuna University.

2.2 Processing plant material and obtaining extracts - The fresh leaves of

Typha australis Schum.& Thonn collected were meticulously selected, discarding all those that presented interruptions in the integrity of the leaf blade, insect attacks, scars from blows, etc. The selected material was subjected to drying in the air oven at 102ºC for 24 hours **[11]**. Ultimately, the parts were ground in a mechanical mill, yielding 881 g of leaves. The dried and powdered leaves were macerated with hexane (440 g of dry powder in 2 L of solvent) and Ethyl acetate (440 g of dry powder in 1.5 L) at room temperature. The leaf powder was macerated with ethyl alcohol (371 g of dry powder in 2 L of solvent). For each procedure, three extractions were performed within a 72-hour interval between each extraction. The extractive solutions obtained were concentrated in a rotary evaporator under reduced pressure. After evaporation of the solvent, the yields were Water (34 g), water-ethanol (50:50) (37 g), n-hexane (36 g), Dichloromethane (37 g) and Ethyl acetate (96 g) extracts of the leaves and the ethanolic extract (91g) **[12]**. The solubility test was carried out to test the ability of the extract to dissolve in the following solvents: Water, water-ethanol (50:50), ethanol, n-hexane, Dichloromethane and ethyl acetate; 5 vials were taken, and 30 mg of the test sample were placed in each one, to which 1 ml of a solvent was added and shaken.

Figure 1: *Typha australis* leaf and extraction process

2.3 Phytochemical analysis of leaf extracts: Using solvents with various degrees of polarity, the analysis was done to acquire the distinct fractions of the extract [Water (A); Water+ Ethanol(B); Ethanol (C); n-hexane (D); dichloromethane (E) and Ethyl acetate (F)]. To identify the secondary metabolites, present in the extract, colouration and precipitation responses persisted. The extracts were subjected to the phytochemical prospecting method **[13-14]** which entails the preliminary determination of the classes of secondary constituents for chemical analysis at the Laboratory of Microbiology and Biochemistry, Acharya Nagarjuna University.

2.3 Extract analysis via high-performance liquid chromatography (HPLC) with a diode array detector (DAD): The ethanolic extract of *T. australis* leaves was examined

at with a High-Performance Liquid Chromatograph (HPLC) model VARIAN 210, diode array detector (DAD), and scanning between 200 and 800 nm. Supelco column C-18 (4.6 mm x 250 mm, 10 µm particle diameter) and pre-column (25 mm x 3 mm) of the same phase as the column. Eluent system gradient methanol/Ac-OH 6% and 2-mM sodium acetate from 5% to 15% methanol (v/v) to 45, from 15% to 30% methanol (v/v) to 55, from 30% to 50% methanol (v/v) to 60, and from 50% to 100% methanol (v/v) to 65, plus 5 minutes to return to the initial condition. The flow rate of the pump is 1 mL/min, and the volume injected is 5µL, λ 325 nm. A 0.45µm microfilter was used to clean the samples. Fourteen standards were injected into the same sample under the same conditions. The standards used were rosmarinic acid, caffeic acid, carnosol, ferulic acid, p-coumaric acid, vanillic acid, syringic acid, vanillin, sinapic acid, luteolin, apigenin, rutin, gallic acid, kaempferol, and quercetin.

2.4 Quantification of phenols: The total phenolic content of the *T. australis* extracts was determined as gallic acid equivalent (mgGAE/g) using Folin-Ciocalteu Reagent (FCR) **[15]**. 1 mg/mL extract solution was mixed with dH_2O (46 mL) and FCR (1 mL) for 3 min. Then 2% Na_2CO_3 (3 mL) solution was added. The mixture was incubated for 2 hours at room temperature and shaken periodically. At the end of the time, absorbance values were determined using the UV-Vis spectrophotometer wavelength reading at 760 nm. Based on a standard curve constructed with different concentrations of gallic acid, the total phenolic in the samples was calculated using the following linear equation ($y = 0.0016x + 0.0659$ with $R^2 = 0.9998$).

2.5 Determination of total flavonoid content: The total amount of flavonoid substances in *T. australis* extracts was determined using the aluminium chloride spectrophotometric method equivalent to quercetin **[15-16]**. According to this method, after mixing 2% $AlCl_3$ (1 mL) with 2 mg/mL plant extract (1 mL), it was left to incubate at room temperature for 10 minutes. At the end of the time, the absorbance of the mixture against the blank was determined at 415 nm. The same procedures were performed for the standard flavonoid quercetin, and the total flavonoid content of the extracts was given as quercetin equivalent (mgQE/g). The total flavonoid content was calculated using the following equation ($y = 0.009x + 0.056$ with $R^2 = 0.9997$), which was built from different concentrations of quercetin standard.

2.6 Minimum Inhibitory Concentration (MIC) of the ethanolic extract: The MIC of the extract was determined using the broth microdilution technique by the guidelines of the Clinical and Laboratory

Standards Institute (CLSI, 2008). The microorganisms tested in the current study are two-gram positive bacteria (*Streptococcus pyogenes* ATCC 12344; *Staphylococcus epidermidis* ATCC 12228) and two-gram harmful bacteria (*Klebsiella pneumoniae* ATCC 13883; *Acinetobacter baumannii* ATCC 27853) and two fungal strains (*Candida dubliniensis* ATCC 44508, *Candida metapsilosis* ATCC 10232). The fungal strains were taken into Yeast Peptone Dextrose broth containing 15% glycerol and stored at -86°C. After dissolving the ethanolic extract in Dimethylsulfoxide (DMSO, SD fine chemicals), serial dilutions with RPMI 1640 broth (Sigma-Aldrich) for yeast and Müller Hinton broth (Hi media) for bacteria were performed. The concentrations were distributed in 96-well microdilution plates (Sigma-Aldrich), starting with 2089µg/mL and ending with µg/mL. Soon after, 100 L of inoculum with a concentration of 0.5 McFarland (10^8CFU/mL) was added to the wells. The microdilution plates were incubated at 35°C for 48 hours (for yeast) and 37°C for 24 hours (for bacteria). Fluconazole (a conventional antifungal) and ampicillin (an antibiotic) were positive controls. The minimum extract concentration (MIC) was defined as the lowest concentration at which microorganisms did not grow after incubation. The experiment was repeated twice **[17]**.

2.7 Antifungal susceptibility tests: To determine the Minimum Inhibition Concentration (MIC) value of *T. australis* leaf extract, the Clinical and Laboratory Standards Institute microdilution reference method was used (CLSI M27-A2) **[18-19]**. *T. australis* leaf extract with an initial concentration of 40 µl/ml. In the study, two-fold dilutions of *T. australis* leaf ethanolic extract at 0.004%, 0.008%, 0.15%, 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1%, and 2% v/v concentrations were prepared in 96-well microplates and using RPMI 1640 medium (100 µl per well). Yeast suspensions prepared previously were also inoculated with 100 µl per well. Columns 11 and 12 were used as positive and negative controls. Microplates were incubated at 37°C for 24 hours, and MIC evaluations were made. The resazurin solution prepared with 0.01% distilled water was sterilized by passing through a 0.22 µm diameter membrane filter **[20]**. After adding 30 µl of resazurin to each well, the plates were left for 1 hour, and the results were evaluated visually. The MIC value was defined as the lowest concentration of oil that inhibited fungal growth compared to the positive control. In order to determine the minimum fungicidal concentration (MFC) values, 0.1 ml was taken from clean and non-growth MIC wells and inoculated into Yeast Peptone Dextrose solid medium **[21]**. MFC was determined as the lowest drug concentration that killed

99.9% of cells. Fluconazole was used as the standard antibiotic against *Candida* species. All experiments were run repeatedly, and the results were averaged. A representative picture showing the microdilution test applied and expressing the MIC results of the Candida isolates is presented in **Figure 2**.

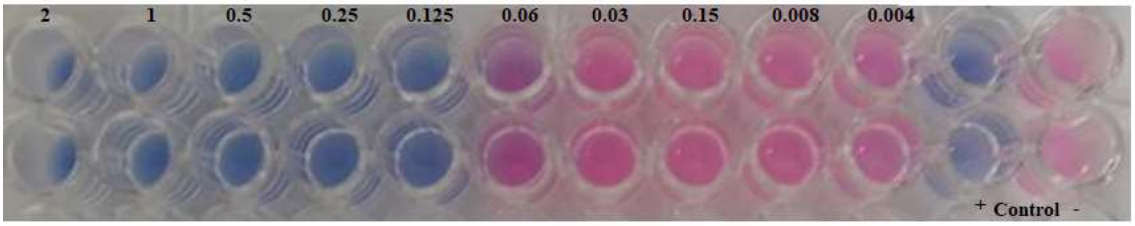

Figure 2: Evaluation of inhibitory effect against *Candida* isolates by liquid microdilution test

2.8. Antioxidant activity evaluation by DPPH free radical antioxidant assay: The antioxidant test with the free radical DPPH (1,1 – diphenyl – 2 picryl – hydroxyl) was performed on the sample using a prepared solution of 0.004% DPPH in methanol. The sample was analysed using different concentrations from 0.2 to 400 µg /mL. To each 0.5 mL of the prepared solution, 1 mL of the DPPH solution was added. For the blank, the sample solubilization solvent (ethanol) was used. Through the absorbances, the percentage of inhibition (PI) was calculated **[22]**. Concentrations and PI were used to obtain the (IC_{50}) of each sample. All assays were performed in triplicate and accompanied by quercetin as a control antioxidant. DPPH is a stable, violet-coloured organic nitrogen radical with 515-517 nm absorption range. The reduction of the DPPH radical is monitored by the decrease in absorbance during the reaction (DPPH changes to yellow when reduced). All tests are performed in triplicate. The data is treated using a linear regression analysis using Microsoft Office Excel 2019.

2.9 Toxicity assessment- *Allium cepa* test: To evaluate toxicity effects, *Allium cepa* seeds were subjected to continuous treatment, and the parameter analysed was the average length of the roots of the seeds (CMR) and the germination index (GI). For this, 50 to 100 seeds of *Allium cepa*, of the bay pear-shaped type, were distributed in glass Petri dishes (100 x 15 mm) with a sheet of filter paper moistened with different concentrations of *T. australis* extract (0.2, 0.3 and 0.5 mg /mL), remaining for 5 days at room temperature. For the negative control (CN), the seeds germinated in distilled water, and for the positive control (CP), in 0.1% Cyclophosphamide (Baxter). The roots were measured using a ruler. The CMR was determined by the mean size of the roots for each sample, and the GI by the mean number of roots germinated per day in each treatment **[23]**.

2.9.1 Evaluation of cytotoxicity: For cytotoxicity evaluation, seeds of *Allium cepa* were subjected to discontinuous treatment. From 50 to 100 seeds of *Allium cepa*, of the pear-shaped bay type, were distributed in glass Petri dishes (100 x 15 mm) with a sheet of filter paper moistened with distilled water at room temperature for 4 to 5 days as substrate. When the roots reached 2 to 4 cm in length, they were treated with different concentrations of the ethanolic extract of *T. australis* (0.2, 0.3 and 0.5 mg /mL). For the negative control (CN), the seeds germinated in distilled water, and for the positive control (CP), in 0.1% Cyclophosphamide (Baxter). After 24 hours, *A. cepa* roots were collected and fixed in Carnoy. To assemble the slides, the roots of *A. cepa* were stained with Schiff's Reagent and 2% acetic carmine, covered with a cover slip, and the slide and cover slip wrapped in filter paper were gently pressed and observed under a microscope. The slides were made using the tips of the roots of germinated seeds in different concentrations, referring to the analysed extract and its controls. The cytotoxic activity analysis involves investigating chromosomal abnormalities (CA) and the mitotic index (MI) in the root cells of *A. cepa* seeds, 5 slides per treatment were analysed, and 1000 cells were counted per slide, totalling 5000 cells per treatment **[24]**.

2.10. Pharmacological study

Carrageenan-induced plantar oedema method: Technique used to assess the anti-inflammatory activity of substances. Initially described by Winter *et al*., (1962 **[25]**. Twenty-five Holtzman rats were used, which, after their acquisition, underwent an adaptation process: for one week, temperature 21 ± 2°C; the cycle of 12 hours of light and 12 hours of darkness; free access to food and water; 12 hours before the start of the trial only access to water. During the experiment nothing to drink or eat. Five groups of five rats were randomly formed, and the treatment corresponding to each group was administered orally **(Table 1)**. Half an hour later, 0.1 ml of 1% carrageenan was applied to the aponeurosis of the right hind paw of each experimental animal **[26]**.

Table 1: Experimental anti-inflammatory treatment.

GROUP	TREATMENT	DOSES
control	Saline solution	1.5 ml
standard	naproxen	50 mg/kg
ethanolic extract	*T. australis*	100 mg/kg
ethanolic extract	*T. australis*	200 mg/kg
ethanolic extract	*T. australis*	500 mg/kg

Paw volume was measured with a digital plethysmometer at 0o'clock; 1; 2; 3; 4; 5 and 6 hours after inducing inflammation **[26]**.

The percentage of inflammation will be determined according to the following formula:

% of inflammation = $\frac{(V_{tx}-V_{t0})}{V_{t0}} \times 100$

Where:

V_{tx} = volume of the inflamed paw at time X.

V_{t0}= normal volume of the paw.

According to the data, the percentages of inflammation are determined.

2.11 Ethical aspects

The outcomes are documented exactly as they were received. The various sources consulted are appropriately acknowledged. Informed consent was not required because we did not intervene in the study variables and limited ourselves to describing the reactions of the substances and plant organic material *In Vitro*. The Bioethics Committee of Nagarjuna University's Faculty of Biochemistry and Pharmacy approved the research protocol (File No:25/2019).

3. RESULTS AND DISCUSSION

The maceration of 750g of dry and ground leaves yielded 105g of dry extract, equivalent to 14%. The solubility analyses yielded the results shown in the following **Table 2**.

3.1 Detection of secondary metabolites

The direct tests' results for identifying metabolites in the different fractions of the ethanolic extract of *T. australis* are presented in **Table 3**.

As indicated in **Table 3**, the major secondary metabolites are Phenols, flavonoids, saponins, leucoanthocyanidins, triterpenes, and tannins. The results support the proposed study hypotheses.

3.1.1 Test of total phenolic compounds

The results of the assay for the quantification of total phenolic compounds from the Folin Ciocateu reagent showed that the leaf extract fraction obtained with Ethanol fraction had the highest index of these compounds, followed closely by the ethanol-water fraction, water extract and fraction of ethyl acetate, as shown in **Figure 3.** The n-hexane fraction showed the lowest number of phenolic compounds. These results corroborate those obtained in a study that preliminarily detected the presence of polyphenols and flavonoids in the extracts of leaves and roots of *T. australis* **[27]**. Regarding the low index in the n-hexane fraction, a study carried out with *T. australis* leaf extracts demonstrated that phenolic compounds are more abundant in the more polar fractions of the extract (Dilshad, *et al*., 2022).

Total flavonoid content For the quantification of total flavonoids, the results obtained showed that the ethanolic fraction of the leaf extract had the highest concentration of flavonoids, followed by the Water-ethanol 50:50 fraction, Water fraction, and Ethyl acetate fraction. However, none of the values were significant, as shown in **Figure 4**.

Again, the n-hexane fraction showed a low amount, this time of flavonoids, confirming

the polar characteristic of these phenolic compounds. The results obtained align with those presented in a study found in the literature that confirmed flavonoids' presence in plant material extracts from *T. australis*. In studies with other plants of the genus Typha, flavonoids are also present, a frequent class of metabolites in plants of this genus **[28]**.

3.1.2 Determination of the phenolic content by HPLC-DAD

Regarding the analysis of the extracts, the evaluation was performed preliminarily with the ethanolic fraction of *T. australis*, due to the lower polarity of the other fractions. Although literature data indicate the presence of phenolic compounds in *T. australis* extracts, there are still no reports about their types. Therefore, the absence of rutin, quercetin and coumarin in the extracts leaves a gap regarding the identity of these phenolic compounds. The verification of the presence of phenolic compounds in HPLC began with recording the standards for later comparison with the extract and fractions, comparing the peaks and their respective retention times. The three-dimensional visualization of the chromatogram associated with the ultraviolet spectrum was a crucial tool in recording the patterns. The quercetin chromatogram showed the major peak at the retention time of 10.827 min.

Chromatograms and UV-vis spectra (UV/Visible Spectroscopy) were not presented for all standards, as it was impossible to relate any standard to a substance. Group 2 of UV spectra indicates that we have 6 substances that belong to the same class of compounds; in this case, it was possible to compare the UV spectrum with that of rutin, indicating that these substances belong to the class of flavonoids (flavonol), these data are in line with according to the literature found for the chemical study of the roots of *T. australis*. From the UV 1, 3, 4 and 5 spectra, it was not possible to determine the class of compounds **(Figure 4)**. Alkhalifawi, *et al* (2017) **[28]** report that the chromatographic analysis of the ethanol extract of *T. australis* indicated the presence of flavonoids such as rutin, quercetin and gallic acid. The flavonoid rutin is widely spread in the plant kingdom and presents important biological activities, such as antioxidant, vasodilator, and anti-inflammatory action.

3.2. Antioxidant activity according to the DPPH method

The correlation between antioxidant activity (%) and the concentration of extract used provided an IC_{50} of 18.67 μg /mL, which is the concentration of ethanolic extract necessary to cause 50% antioxidant activity. This concentration was significant when compared to commercial antioxidants such as ascorbic acid (IC_{50}=2.15 μg /mL) and Butyl hydroxytoluene (BHT) (IC_{50}=5.37 μg/mL). Scientific evidence **[27]**

allows us to state that the antioxidant property of vegetables is mainly due to their phenolic compounds. Babbar, *et al* (2011) **[29]** found a significant and positive correlation (r^2=0.66, p<0.05) between antioxidant activity and total phenol content in 6 important fruit residues. Flavonoids present, in the human body, a series of pharmacological activities, among which the most important is their antioxidant property in the treatment of various pathologies such as heart disease, arteriosclerosis, cancer, neurodegenerative diseases, ageing, and others related to oxidative stress. This result allows prognosing that the antioxidant activity of *T. australis* is due to the presence of phenols and flavonoids, determined in the phytochemical screening carried out in this work.

The literature **[29-30]** shows that the antioxidant and antifungal activities may be related to the type of phenolic compounds present in a wide variety of plant materials, a fact that led to the possibility of associating them with antifungal activity on *Candida* isolates, evaluated in this work, with the presence of phenols. Researchers are looking for natural substances with antioxidant properties that can work alone or with other additives to stop food from going bad due to oxidation and reduce the use of synthetic antioxidants and preservatives.

3.3. Calculation of the Extract's Minimum Inhibitory Concentration (MIC)

The antimicrobial activity of plant extract is good if its MIC is less than 100µg/mL, median if it's between 100 and 500µg/mL, weak if it's between 500 and 1000µg, and inactive if it's over 1000µg. Based on these criteria, the ethanolic extract of the *T. australis* leaf obtained good results against the microorganisms studied, showing good action against *Candida metapsilosis* (65µg/mL), *Staphylococcus epidermidis* (4µg /mL) and *Klebsiella pneumonia* (8µg/mL); medium effect against *Streptococcus pyogenes* (522µg /mL) and inactive action against *Candida dubliniensis* (1044µg /mL) **(Table 5)**.

3.4 *Allium cepa* test

The cytotoxic effects of *T. australis* extract on meristematic cells of *Allium cepa* root were evaluated in discontinuous treatment at different concentrations (0.2, 0.3, and 0.5 mg /mL) after germination. With the results of this treatment, it was verified that the mitotic (MI) and alteration (IA) indices did not present significant differences compared to the positive control group, suggesting that the *T. australis* extract does not interfere with cell division **(Figure 5)**.

The values of the root length of *Allium cepa* seeds obtained in the continuous treatment showed inhibition in the development of the roots exposed in all

concentrations in the ethanolic extract of the leaves of *T. australis*. An agent can be considered toxic when it promotes a reduction greater than 50% in the germination index (GI) of *Allium cepa* seeds about the negative control. The germination rates of *Allium cepa* seeds under continuous treatment in the ethanol extract of *T. australis* leaves were 59.09% at a concentration of 0.2 mg /mL, 51.04% at 0.3 mg /mL , 41.86% in 0.5 mg /mL. These results indicate that there was a reduction in the germination index in all tested concentrations when compared with the negative control, which had a GI of 56.97% and in the positive control, it was 60.63%, and it can be seen that the index was affected proportionally to the increase in extract concentration **(Figure 6)**. These data demonstrate that the tested concentrations are not close to the limit for *T. australis* toxicity in plant cells.

The *Allium cepa* test was used by Akinboro and Bakare in 2007 **[31]** to find out how dangerous the aqueous extract of five medicinal plants was. At 2.6 and 0.8% concentrations, water extracts *of Azadirachta indica* and *Morinda lucida* showed little root growth. Even though the positive control (cyclophosphamide) changed the mitotic index, it did not change how fast the roots of Allium cepa seeds grew. The germination test with plants is a widely used model to evaluate the allelochemical potential of plant extracts or isolated substances, and one of the predicted effects when a certain compound interferes with cellular functioning is the alteration in the germination index of the seeds, revealing toxic action and cytotoxic.

3.5. Pharmacological study

Table 6 presents the values as the mean of the millilitre inflammation when evaluating the hind paw in each experimental group. Some data concerning the control (mean difference) at the 0.05 level are significant. The values are presented in **Table 7** below as the percentage of inflammation during the testing process. The following results are obtained: The control group (only received carrageenan) shows a greater inflammation volume, unlike the rest that received treatment (naproxen and extract). The treatment with naproxen produced a greater anti-inflammatory effect than the ethanolic extract of *T. australis*. Of the extracts, the one administered at a dose of 500 mg/kg had the greatest effect, as evidenced in the following **Tables 6, 7 and Figures 7, 8**.

The following linear graph **(Figure 7)** shows the evolution of oedema in each study group in greater detail. The control group developed a greater volume of oedema; in the same way, it is evident that the standard group (naproxen) had a smaller volume.

Table 2: Solubility of the ethanolic extract of the leaves of *T. australis*

SOLVENTS	RESULTS
Water	+
Water-ethanol 50:50	++
Ethanol	+++
n-hexane	++
Ethyl acetate	+++
Where, slightly soluble (+), soluble (++), very soluble (+++)	

Table 3: Preliminary phytochemical progress of the ethanolic extract from the leaves of *T. australis*.

FRACCIÓN	METABOLITE	Result
A	Tannins, phenols	+++
	flavonoids	++
B	steroids	++
	triterpenes	
	Anthraquinone	-
C	steroids	+
	triterpenes	+
	alkaloids	+
D	flavonoids	+
	Leucoanthocyanidins	++
	alkaloids	-
E	Tannin	+
	flavonoids	+++
	Leucoanthocyanidins	+
	Anthraquinone	-
F	saponins	+++

Legend: No evidence of presence (-); Presence of traces (+); Moderate presence (++); Abundant presence (+++)

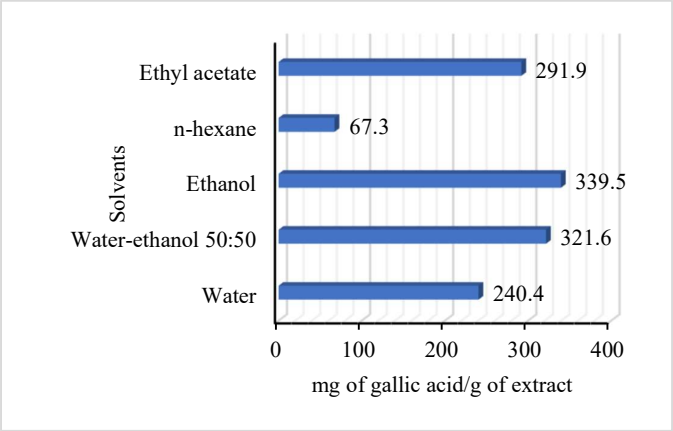

Figure 3: Quantitative test results for the presence of total phenols

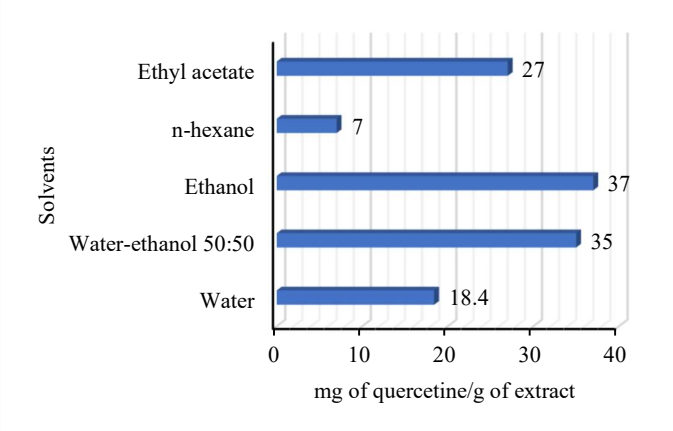

Figure 4: Results of the quantitative test for the presence of flavonoids

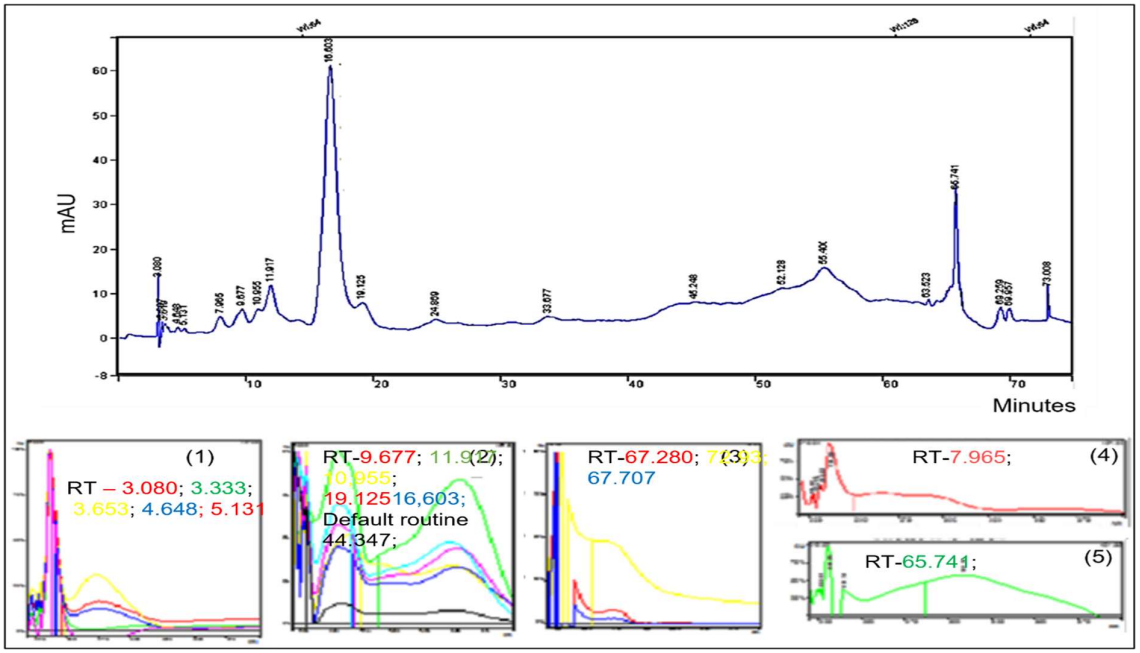

Figure 4: Chromatographic profile and UV spectra obtained via analytical HPLC of the ethanolic extract of *T. australis* leaves

Table 5: MIC, MFC, and MBC in µg /mL of the ethanolic extract of leaves of *T. australis*

	T. australis (leaf extract)		Ampicillin*	fluconazole*
Microorganisms	**MIC**	**MFC/MBC**	**MIC**	**MIC**
***Candida dubliniensis* ATCC 44508**	**1044**	**2089**	**0**	**33**
***Candida metapsilosis* ATCC 10232**	**65**	**522**	**0**	**16**
***Staphylococcus epidermidis* ATCC 12228**	**4**	**4**	**1**	**-**
***Klebsiella pneumoniae* ATCC 13883**	**8**	**-**	**8**	**-**
***Acinetobacter baumannii* ATCC 27853**	**-**	**-**	**1**	**-**
***Streptococcus pyogenes* ATCC 12344**	**522**	**-**	**131**	**-**

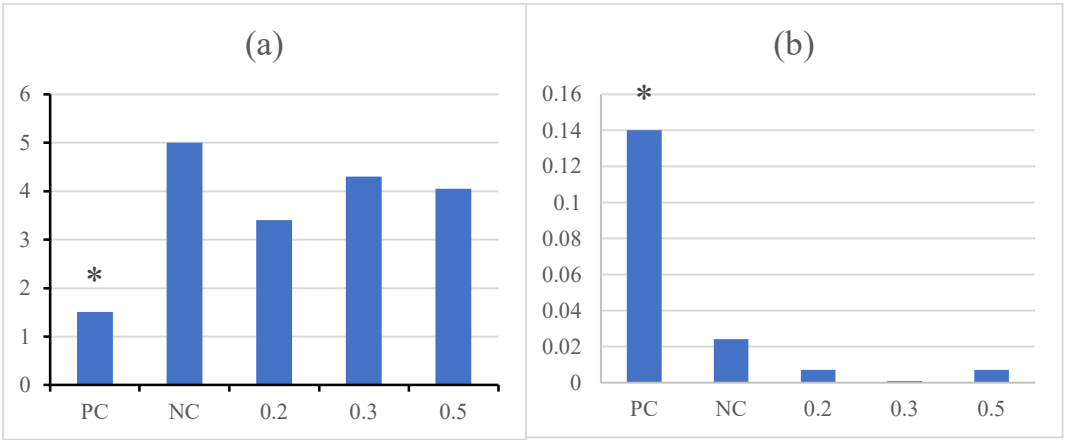

Figure 5: (a) Mean mitotic index and (b) alteration index of *Allium cepa* seeds in response to discontinuous treatment with ethanolic extract of *T. australis* leaves submitted to treatments 0.2; 0.3; 0.5 mg /mL, NC and PC. *Statistically significant values of the positive control concerning the concentrations by the Mann-Whitney test p<0.05.NC: Negative control; PC: Positive Control

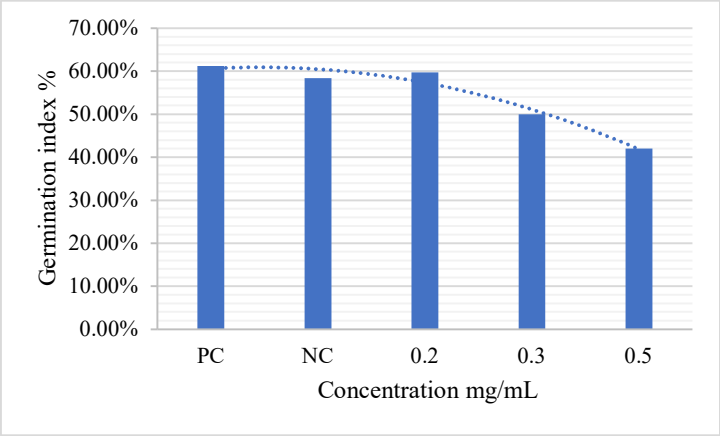

Figure 6: Germination index of *A. cepa* seeds in response to continuous treatment with ethanolic extract of *T. australis* leaves at concentrations of 0.2, 0.3 and 0.5 mg /mL, negative (NC) and positive (PC) control

Table 6: Evolution of inflammation

Treatment	T_0	T_1	T_2	T_3	T_4	T_5	T_6
carrageenan	1.36	1.71	2.15	2.5	2.45	2.22	2.01
Naproxen	1.28	1.37*	1.40*	1.49*	1.58*	1.48*	1.42*
T. australis 50mg/kg	1.29	1.68	1.97	1.94*	1.81*	1.57*	1.47*
T. australis 200mg/kg	1.44	1.56	1.94	2.06*	1.86*	1.82*	1.75*
T. australis 500mg/kg	1.44	1.62	1.85	1.76*	1.78*	1.83*	1.78

A significant difference exists between the means concerning the control group using an ANOVA with post hoc Dunnett's T-tests (<control)

Table 7: Evolution of oedema in percentage

Treatment	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Control	0%	25%	58%	83%	80%	63%	47%
Naproxen	0%	7%	10%	16%	24%	15%	11%
T. australis 50mg/kg	0%	30%	53%	51%	40%	22%	14%
T. australis 200mg/kg	0%	9%	35%	43%	30%	27%	22%
T. australis 500mg/kg	0%	12%	28%	22%	24%	27%	23%

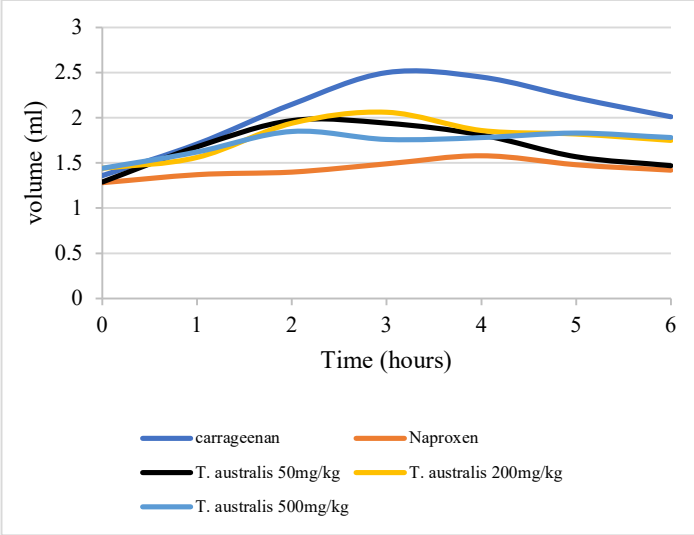

Figure 7: Evolution of oedema concerning the different treatments in each experimental group

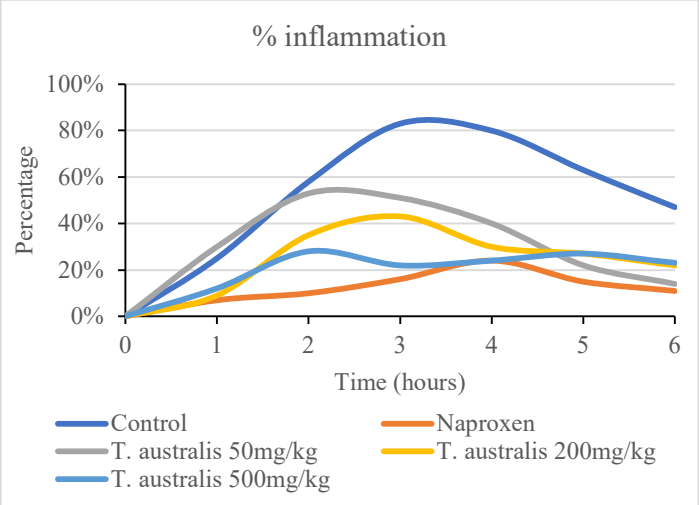

Figure 8: Evolution of oedema in percentage

According to the phytochemical study, the plant species *T. australis* presents steroids, triterpenes, leucoanthocyanidins, flavonoids, tannins, and saponins. There is scientific evidence of flavonoid-type secondary metabolites in the plant species *T. australis* and plants of the same family. For example, Dilshad, *et al* (2022). also determine these types of metabolites in the ethanolic extract of the leaves of the problem plant. *C. metapsilosis* was the species that obtained the best result among the evaluated yeasts. This yeast has great medical relevance and demonstrates intrinsic resistance to Fluconazole, considered a standard antifungal and one of the most prescribed by doctors **[32]**. *Candida metapsilosis* is an occasional hospital pathogen, particularly in patients with malignant haematological diseases and undergoing bone marrow transplantation. Some authors have reported increased fungemia caused by *C. metapsilosis* in neutropenic patients exposed to prolonged treatment with Fluconazole **[33]**. The result obtained in our study for *Staphylococcus epidermidis* and *Klebsiella pneumoniae* was considered good, as they are Gram-negative bacteria whose cell wall structure favours more excellent resistance to antimicrobials. Treating infections caused by *S. epidermidis* is challenging due to its intrinsic resistance to several antibiotics. This resistance has been highlighted by the rapid decline in therapeutic options available for treating infections caused by this bacterial species. *Klebsiella pneumoniae* is one of the main causes of human infectious diseases and is the most frequent etiological agent of primarily respiratory problems. The resistance acquired by the bacteria *K. pneumoniae* is due to the inappropriate use of antimicrobials and the administration in sub-therapeutic doses; this means that only

the most resistant bacterial agents are selected and do not contain the infection. The MICs of the ethanolic extract of the *T. australis* leaf were considered good for the microorganisms tested since the values of the concentrations that inhibited them were much lower compared to the study carried out by Wei, *et al*. (2021) **[39]** with a species of the genus Typha. These authors report that the antimicrobial activity of the ethanolic extract of the root of *Typha angustifolia.* showed MICs for *K. pneumoniae*, *Streptococcus pyogenes*, *S. epidermidis* of >25 mg /mL, 4 mg /mL and 8 mg /mL, respectively.

Khan, *et al*. (2017) **[34]** reported antimicrobial activity against *Candida metapsilosis* of ethanolic extracts of *Azadiracta indica* with a MIC of 33 µg /mL, Chekuri, *et al*. (2018) **[35]** tested extract fractions from *Acalyphaindica* L observed that *K. pneumoniae* was sensitive to the ethyl acetate fraction at a concentration of 256 µg /mL (MIC), *Streptococcus pyogenes* was sensitive to crude hydroethanolic extract (512 µg /mL) and the butanoic (512 µg /mL) and chloroform (256 µg /mL) fractions. Compared to those obtained in this research, these results make it possible to consider that *T. australis* has good antimicrobial potential compared to other popularly used medicinal plants. In the discontinuous treatment, the ethanolic extract of *T. australis* leaves did not change the division index. It did not show an increase in chromosomal alterations about the negative and positive control, which indicates the absence of genotoxicity and mutagenicity.

The results found by Askin Çelik & Aslanturk (2006) **[36]** indicate that the aqueous extract of leaves of *Plantago lanceolata* also does not have a genotoxic effect. The alterations found in the different concentrations used in the discontinuous treatment were bridge, chromosome, and micronucleus loss. In the negative control, alterations such as chromosome breakage and bridge were found. In contrast, in the positive control, it was possible to observe a greater number of alterations, such as binucleated and multipolar cell, chromosome bridge, micronucleus and C-metaphase. The control group developed oedema of greater volume because no anti-inflammatory treatment was administered. The standard drug naproxen had a greater effect, possibly due to its mechanism of action against prostaglandin synthesis by inhibiting cyclooxygenases. The extract administered in the highest concentration (500 mg/kg) was the one that showed the best effect. However, the three doses (also 50 and 200 mg/kg) exerted significant effects ($p<0.05$) compared to the control from the third hour onwards. The anti-inflammatory effect of the problem plant may be due to its secondary metabolites,

including flavonoids, saponins **[10, 37]**. According to consulted literature, these substances produce this effect by inhibiting the metabolism of arachidonic acid **[38]**. In a study of another plant species (*Typha angustata*) that belongs to the same family as our problem plant, Rizwana *et al*. (2022) **[37]** concluded that the inflorescence of this plant species has significant anti-inflammatory activity.

4.CONCLUSION

In medicine, food, and pharmacy, there has been a lot of interest in natural compounds from plants in the past few years. The antibacterial effect of the obtained compounds reveals that these compounds can be used in food, medicine, pharmacy, agriculture and veterinary. According to the phytochemical study, the ethanolic extract of the leaves of *T. australis* presents steroids, triterpenes, leucoanthocyanidins, flavonoids and saponins. This study revealed the antibacterial effect of ethanol extract from *T. australis* leaves on four different *T. australis* bacteria. Ethanol extract obtained from *T. australis* leaves showed the highest antibacterial effect among the other extract used in the study. On the other hand, antifungal drugs are much more limited than antibacterials; Although Fluconazole, frequently used in treatment, is very effective, its high toxicity limits its use. Our research results reveal that cardamom has a potent antifungal activity with low MIC values on *Candida* isolates. The ethanolic extract of the leaves of *T. australis* presents antioxidant activity according to the DPPH method with an IC_{50} of 18.67 µg/ml. The ethanolic extract of the leaves of *T. australis* presents anti-inflammatory activity according to the carrageenan-induced plantar oedema method with a better anti-inflammatory effect at a dose of 500 mg/kg in 2-month-old male albino rats. The ability of the sample to reduce the DPPH radical showed that it had antioxidant properties. Despite the growing use of medicinal plants for therapeutic purposes, it is essential to prove their pharmacological effect. The results of this study indicate the absence of toxic effects from the ethanolic extract of *T. australis* leaves, as it does not interfere with cell division. The *Allium cepa* test is a great biomarker for chromosomal changes that can warn people who use medicinal teas without thinking about it. Even though the biotest is the first test for toxicity, it shows how important scientific discoveries and new versions of the test are. These can show many ways to use the test instead of testing on animals.

REFERENCES

[1] Dal Cero, Maja, Reinhard Saller, Marco Leonti, and Caroline S. Weckerle. (2023) Trends of Medicinal Plant Use over the Last 2000 Years in Central Europe. Plants, 12(1), 135..

[2] Nortjie, Elvino, Moses Basitere, Doice Moyo, and Pardon Nyamukamba. (2022) Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review, Plants, 11 (15), 2011.

[3] Martemucci, Giovanni, Ciro Costagliola, Michele Mariano, Luca D'andrea, Pasquale Napolitano, and Angela Gabriella D'Alessandro. (2022) Free Radical Properties, Source and Targets, Antioxidant Consumption and Health, Oxygen, 2 (2), 48-78.

[4] Martínez-Martínez, Joana Guadalupe, Stephanie Rosales-Loredo, Alejandro Hernández-Morales, Jackeline Lizzeta Arvizu-Gómez, Candy Carranza-Álvarez, José Roberto Macías-Pérez, Gisela Adelina Rolón-Cárdenas, and Juan Ramiro Pacheco-Aguilar. (2023) Bacterial Communities Associated with the Roots of *Typha* spp. and Its Relationship in Phytoremediation Processes, Microorganisms, 11(6), 1587.

[5] Chakraborty M, (1996) A survey of the monocot flora of West Bengal. Journal of Economic and Taxonomic Botany, 20 (1), 131–133.

[6] Minkina, T.M., Fedorenko, G.M., Nevidomskaya, D.G. *et al.* (2021) Bioindication of soil pollution in the Don River delta and the Taganrog Bay coast with heavy metals based on anatomical, morphological and biogeochemical studies of macrophytes. Environ Geochem Health, 43, 1563–1581.

[7] Nayak S K, Satapathy K.B. (2015) Diversity uses and origin of invasive alien plants in Dhenkanal district of Odisha, India. International Research Journal of Biological Sciences, 4 (2), 21-27.

[8] Bandaranayake, W. M. (1998) Traditional and medicinal uses of mangroves, Mangroves and Salt Marshes, 2 (3), 133–148

[9] Sezik, E. Yeşİlada, M. Tabata *et al*., (1997) Traditional medicine in Turkey Viii. Folk medicine in East Anatolia, Erzurum, Erzíncan, Ağri, Kars, Iğdir provinces, *Economic Botany*, 51, 3, 195–211.

[10] Dilshad R, Khan KU, Saeed L, Sherif AE, Ahmad S, Ovatlarnporn C, Nasim J, Hussain M, Ghalloo BA, Basit A, Mukhtar I. (2022). Chemical Composition and Biological Evaluation of *Typha domingensis* Pers. to Ameliorate Health Pathologies) *In Vitro* and *In Silico* Approaches. Biomed. Res Int., 1-16.

[11] Krakowska-Sieprawska, Aneta, Anna Kiełbasa, Katarzyna Rafińska, Magdalena Ligor, and Bogusław Buszewski. 2022 Modern Methods of Pre-Treatment of Plant Material for the Extraction of Bioactive Compounds, Molecules, 27 (3), 730.

[12] Zhang, Huijun, Yushu Yuan, Xiuxiu Zhu, Runzhe Xu, Huishan Shen, Qian Zhang, and Xiangzhen Ge. (2022) The Effect of Different Extraction Methods on Extraction Yield, Physicochemical Properties, and Volatile Compounds from Field Muskmelon Seed Oil, Foods, 11 (5), 721.

[13] Vamanu, Emanuel. (2022), Bioactive Compounds from Natural Products: Separation, Characterization, and Applications, *Applied Sciences* 12 (8), 3922

[14] Colegate, S.M. & Molyneux, R.J. (2007) Bioactive natural products: Detection, isolation, and structural determination, second edition. Boca Raton, FL : CRC Press, c2008.

[15] Kalpoutzakis Eleftherios, Theodoros Chatzimitakos, Vassilis Athanasiadis, Sofia Mitakou, Nektarios Aligiannis, Eleni Bozinou, Olga Gortzi, Leandros A. Skaltsounis, and Stavros I. Lalas. (2023) Determination of the Total Phenolics Content and Antioxidant Activity of Extracts from Parts of Plants from the Greek Island of Crete, Plants, 12 (5), 1092

[16] Al-Dalahmeh Yousef, Nezar Al-Bataineh, Sara S. Al-Balawi, Jamil N. Lahham, Idrees F. Al-Momani, Mohammed S. Al-Sheraideh, Abdulraouf S. Mayyas, Sultan T. Abu Orabi, and Mahmoud A. Al-Qudah. (2022). LC-MS/MS Screening, Total Phenolic, Flavonoid and Antioxidant Contents of Crude Extracts from Three Asclepiadaceae Species Growing in Jordan, Molecules, 27 (3), 859.

[17] Arsene, Mbarga M. J., Podoprigora I. Viktorovna, Marukhlenko V. Alla, Morozova A. Mariya, Goriainov V. Sergei, Esparza Cesar, Anyutoulou K. L. Davares, Kezimana Parfait, Kamgang N. Wilfrid, Tuturov S. Nikolay, and et al. (2022). Optimization of Ethanolic Extraction of *Enantia chloranta* Bark, Phytochemical Composition, Green Synthesis of Silver Nanoparticles, and Antimicrobial Activity, Fermentation, 8 (10), 530.

[18] Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing*, 28th Ed., CLSI Supplement M100, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018, ISBN1 978-

1-68440-066-9. [Print], ISBN2 978-1-68440-067-6.

[19] Lavogina, Darja, Helen Lust, Maris-Johanna Tahk, Tõnis Laasfeld, Hans Vellama, Naila Nasirova, Markus Vardja, Kattri-Liis Eskla, Andres Salumets, Ago Rinken, and et al. (2022) Revisiting the Resazurin-Based Sensing of Cellular Viability: Widening the Application Horizon. Biosensors,12 (4), 196

[20] Alexander B.D., Byrne T.C., Smith K.L., Hanson K.E., Anstrom K.J., Perfect J.R., Reller, L.B. (2007) Comparative evaluation of Etest and sensititre yeastone panels against the Clinical and Laboratory Standards Institute M27-A2 reference broth microdilution method for testing *Candida* susceptibility to seven antifungal agents. J. Clin. Microbiol., 45, 698–706

[21] Gucwa K., Milewski S., Dymerski T., Szweda P. (2018) Investigation of the Antifungal Activity and Mode of Action of *Thymus vulgaris*, *Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum*, and *Eugenia caryophyllus* Essential Oils. Molecules, 23, 1116.

[22] Baliyan, Siddartha, Riya Mukherjee, Anjali Priyadarshini, Arpana Vibhuti, Archana Gupta, Ramendra Pati Pandey, and Chung-Ming Chang. (2022) Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*, Molecules, 27 (4), 1326.

[23] Vujčić Bok, Valerija, Marko Gerić, Goran Gajski, Sanja Gagić, and Ana-Marija Domijan. (2023) Phytotoxicity of Bisphenol A to *Allium cepa* Root Cells Is Mediated through Growth Hormone Gibberellic Acid and Reactive Oxygen Species, Molecules, 28 (5), 2046.

[24] Bhuia, Md. Shimul, Md. Sajjad Hossain Siam, Md. Riat Ahamed, Uttam Kumar Roy, Md. Imran Hossain, Md. Rokonuzzman, Tawhida Islam, Rezoan Sharafat, Mehedi Hasan Bappi, Md. Nayem Mia, and et al. (2023) Toxicity Analysis of Some Frequently Used Food Processing Chemicals Using *Allium cepa* Biomonitoring System, Biology, 12 (5), 637

[25] Mancipe, Juan C., Pedro Vargas-Pinto, Oscar E. Rodríguez, Paola Borrego-Muñoz, Iovana Castellanos Londoño, David Ramírez, Luis G. Piñeros, María Camila Mejía, and Luis M. Pombo. (2023) Anti-Inflammatory Effect of Izalpinin Derived from *Chromolaena leivensis*: λ-Carrageenan-Induced Paw Edema and

In Silico Model, Molecules, 28 (9), 3722.

[26] Rani, Abida, Muhammad Uzair, Shehbaz Ali, Muhammad Qamar, Naveed Ahmad, Malik Waseem Abbas, and Tuba Esatbeyoglu. (2022) *Dryopteris juxtapostia* Root and Shoot: Determination of Phytochemicals, Antioxidant, Anti-Inflammatory, and Hepatoprotective Effects, and Toxicity Assessment, Antioxidants, 11 (9), 1670.

[27] Reddy P., Moinulla Sk., Bhattacharjee Ronit. (2023) Antioxidant Activity, Total Phenolic and Total Flavinoid Contents of the Leaf Extracts of the Most Common weeds *Mirabilis jalapa* and *Typha domingensis,* International Conference on Recent Advances in Allied Health and Biological Sciences Research 18 & 19 November 2022, Reva University, India.

[28] Alkhalifawi I., Alazawii Y. Al-Fartosi K., Mousa Hind. (2017) Physicochemical, phytochemical profiling and biological activities of leaves extract of Bardy (Typha domingensis Pers.) from Al-Chibayish marshes in southern Iraq. Global Proceedings Repository, 128-143.

[29] Babbar N., Oberoi H., Uppal D., Patil R. (2011) Total phenolic content and antioxidant capacity of extracts obtained from 6 important fruit residues. Food Research International, 44, 391-396.

[30] Cosme, Patricia, Ana B. Rodríguez, Javier Espino, and María Garrido. (2020) Plant Phenolics), Bioavailability as a Key Determinant of Their Potential Health-Promoting Applications, Antioxidants, 9 (12), 1263.

[31] Akinboro A, Bakare AA. (2007) Cytotoxic and genotoxic effects of aqueous extracts of five medicinal plants on *Allium cepa* Linn. J. Ethnopharmacol., 112(3), 470-475.

[32] Mobin M, Szeszs MW, Takahashi JP, Martins M, de Hippólito DDC, Porto JCS, Teles JB, de Lima SG, Melhem MSC. (2018) Antifungal Susceptibility of *Candida* Species Isolated from Horticulturists with Onychomycosis in Piauí, Brazil. Iran. J. Public. Health., 47(12),1816-1821

[33] Pinhati H.M.S., Casulari L.A., Souza A.C.R. *et al.* (2016) Outbreak of candidemia caused by fluconazole resistant *Candida parapsilosis* strains in an intensive care unit. BMC. Infect. Dis., 16, 433

[34] Khan S., Imran Md., Pindari N. (2017) Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant

Candida spp. Bioinformation. 13, 67-72

[35] Chekuri S., Arun B, Sompaga S., Panjala S., Rani R. (2018) Evaluation of Anti-Microbial and Anti-Fungal Activity of *Acalyphaindica* L., Leaf Extract. International Journal of Pharmacognosy and Phytochemical Research, 10(1), 48-51

[36] Askin celik, Tülay, Aslantürk, Özlem. (2006) Anti-mitotic and anti-genotoxic effects of Plantago lanceolata aqueous extract on *Allium cepa* root tip meristem cells. Biologia, 61, 693-697.

[37] Rizwana Dilshad, Kashif-ur-Rehman Khan, Saeed Ahmad, Hanan Aati, Jawaher Al-qahtani, Asmaa Sherif, Musaddique Hussain, Bilal Ahmad Ghalloo, Humna Tahir, Abdul Basit, Maqsood Ahmed. (2022) Phytochemical profiling, in vitro biological activities, and in-silico molecular docking studies of *Typha domingensis*, Arabian Journal of Chemistry, 15 (10), 104133

[38] Chai Tsun-Thai, Mohan Mirohsha, Ong Hean-Chooi, Wong Fai-Chu. (2014) Antioxidant, Iron-chelating and Anti-glucosidase Activities of *Typha domingensis* Pers (Typhaceae). Tropical Journal of Pharmaceutical Research, 13, 67-72.

[39] Wei Huan, Shi Yuqi, Yuan Zhixiang, Huang Zhinan, Cai Fuhong, Zhu Jingfeng, Zhang, Wanwan, Li Jia, Xiong Qingping, Wang Yunpeng, Wang Xiaoli. (2021) Isolation, Identification, and Anti-Inflammatory Activity of Polysaccharides of *Typha angustifolia.* Biomacromolecules, 22(6), 2451-2459.