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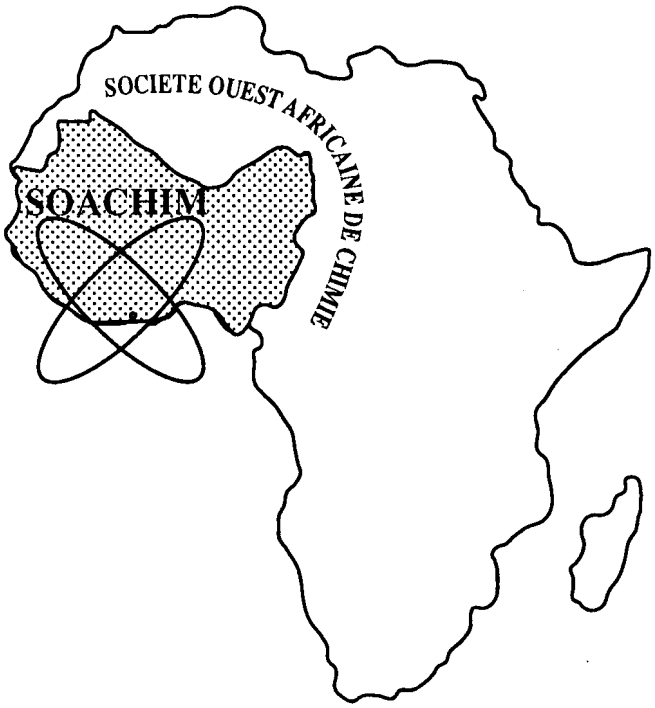

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Phytochemistry and evaluation of the antioxidant activities of the essential oil and leaf extracts of the plant *Melaleuca leucadendra (L)* L

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Abstract : This study explores the phytochemical compounds and antioxidant activities of the essential oil and aqueous, methanolic, and hydroethanolic extracts of *Melaleuca leucadendra (L) L*. A phytochemical screening was carried out to identify the main classes of compounds present in the extracts. Assays of polyphenols, flavonoids, and hydrolyzable tannins were carried out on the essential oil and the various extracts to quantify these bioactive compounds. Antioxidant activity was assessed using the DPPH method. Phytochemical screening results showed that the extracts were rich in secondary metabolites, mainly polyphenols, flavonoids, alkaloids, and tannins. Quantitative analysis of hydrolyzable polyphenols, flavonoids and tannins revealed considerable contents in varying proportions. Evaluation of antioxidant potential by the DPPH method revealed very high antioxidant power, with IC50s as follows: essential oil (0.15 mg/mL), aqueous (0.123mg/mL), methanolic (0.156 mg/mL) and hydro-ethanolic (0.142 mg/mL). These findings suggest that *Melaleuca leucadendra (L) L* could be a promising source of natural antioxidants, offering interesting prospects for the development of natural pharmaceutical and cosmetic products.

Keywords: *Melaleuca leucadendra (L) L*, phytochemical screening, assay, antioxidant activity, DPPH, IC50.

Phytochimie et évaluation des activités antioxydantes de l'huile essentielle et d'extraits desfeuilles de la plante *Melaleuca leucadendra (L) L*

Résumé : Cette présente étude explore les composés phytochimiques et évalue les activités antioxydantes de l'huile essentielle et des extraits aqueux, méthanolique et hydro-éthanoliques des feuilles de *Melaleuca leucadendra (L) L*. Un criblage phytochimique a été réalisé pour identifier les principales classes de composés présents dans les extraits. Les dosages des polyphénols, des flavonoïdes et des tannins hydrolysables ont été effectués sur les différents extraits pour quantifier ces composés bioactifs. L'activité antioxydante a été évaluée à l'aide de la méthode DPPH. Les résultats de criblage phytochimique ont montré la la présence des polyphénols, flavonoïdes, alcaloïdes et des tannins. L'analyse quantitative des polyphenols, flavonoïdes et tannins hydrolysables a révélé des teneurs considérables avec des proportions différentes. L'évaluation du potentiel antioxydant par la méthode DPPH a mis évidence un pouvoir antioxydant très important avec des CI 50 comme suit : huile essentielle (0,15 mg/mL), aqueux (0,123 mg/mL), méthanolique (0,156 mg/mL) et hydro-éthanolique (0,142 mg/mL). Ces résultas suggèrent que le *Melaleuca leucadendra (L) L* pourrait être une source prometteuse d'antioxydants naturels offrant des perspectives intéressantes pour le développement de produits pharmaceutiques et cosmétiques naturels.

Mots clés : *Melaleuca leucadendra (L) L*, screening phytochimique, dosage, activité antioxydante, DPPH, CI 50 .

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1. Introduction

Plants have long played an important role in human life. All ancient civilizations have used wild and cultivated plants for nourishment, defense, and treatment.[1] Recourse to traditional medicine is a very common practice in the countryside and even in cities. The World Health Organization (WHO) estimates that 80% of the population in developing countries use medicinal plants to combat a range of illnesses and provide health care, relying mainly on plant extracts to meet their needs.[2]

Today, we increasingly understand that the active principles of medicinal plants are often linked to the products of secondary metabolites, which are widely used as preventive, anti-inflammatory, antimicrobial, antiseptic, and diuretic agents but essentially as antioxidants that defend against oxidative stress.[3] Aromatherapy is attracting a growing number of patients, and scientific publications on the therapeutic properties of essential oils continue to flourish.[4]

Indeed, medicinal plants represent an inexhaustible source of traditional and effective remedies, thanks to active principles such as alkaloids, phenolic acids, flavonoids, heterosides, saponins, and coumarins.[5] These compounds have multiple uses in the food, pharmaceutical, and cosmetics industries.[6]

In this context, we are interested in studying *Melaleuca leucadendra (L) L*. This plant belongs to the Myrtaceae family (140 genera and 3,000 species), which are highly aromatic trees or shrubs. Therefore, we propose a bibliographical review to present the state of botanical, phytochemical, and biological knowledge, followed by a presentation of the experimental part involving phytochemical screening, assays, and evaluation of the antioxidant activity of the extracts.

2. Botanical description

Melaleuca leucadendra is a tree in the Myrtaceae family (Figure 1), native to Australia and Indonesia. The variant spelling *Melaleuca leucadendron* was used in the past. Since 1966, it has been banned [7], as it contravenes the international code of botanical nomenclature.

Until Blake's taxonomic revision of the *Melaleuca leucadendra* complex based on floral morphology and indumentum (hair) type, the name was used broadly to refer to several closely related broad-leaved Melaleuca species. This species has often been confused with closely related broad-leaved Melaleuca viridiflora, Melaleuca quinquenervia, or Melaleuca cajuputi (cajeput). Australian botanist Lyn Craven [8], a specialist in Australian melaleucas, considers it somewhat ironic that these prominent species in the Australian landscape, known locally as “broad-leaved paperbark” and so useful for their wood, have given botanists such a hard time classifying them.

Figure 1: Myrtaceae family [7]

The Myrtaceae family comprises some 140 genera and over 3,000 species, many of them aromatic. The botanical classification of *Melaleuca leucadendra* is given in the following table:

Table I: Botanical classification of *Melaleuca leucadendra* Vernacular name: Wolof (Niaouli)

Kingdom	Plantea
Subdomain	Tracheobiota
Division	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	Melaleuca

M. leucadendra is native to Australia (Western Australia, Northern Territory, Queensland), the Moluccas on the eastern islands of Indonesia.[9] It grows wherever groundwater is abundant, along northern Australia's watercourses, swampy areas, and tropical rainforests.[10]They tolerate acid and infertile soils. Adventitious root formation is observed when they grow in flooded areas. The trees also show good tolerance to fire [11]. It is also grown in parks and along streets. *Melaleuca leucadendra (L) L*. is a tall tree, up to 40 m high, with whitish bark, formed of several layers exfoliating in broad bands (figure 2).[12] Because of its slender twigs and drooping leaves, it is known as “weeping tea tree”, Melaleuca pleureur. Young twigs are covered with whitish pubescence.[10]

The leaves are narrowly lanceolate (Fig. 2), very long (7.5 to 27 cm long, i.e., 3.5 to 16 times longer than they are wide), and among the longest in the

genus. They are crossed by 5 longitudinal veins.[12] Inflorescences are cylindrical spikes, grouping flowers by three (3) months. The white flowers have numerous stamens, 7 to 16 mm long, grouped in 5 clusters opposite the petals. The hypanthium is glabrous, while those of cajeput and niaouli are pubescent. The fruit is a woody, glabrous capsule over 4 mm in diameter.[12]

3. Therapeutic uses

Melaleucadra was introduced to India and grows in gardens and parks as an ornamental tree. Fresh leaves yield an oil used as an expectorant for chronic laryngitis and bronchitis, and as a carminative, overdoses cause gastrointestinal irritation. The plant is also used as an anthelmintic, particularly against roundworms.[13]

In Indonesia, the oil of the plant is used in herbal remedies, including antiseptics, antispasmodics, antinevralgics, antihumanisms, and in the manufacture of cosmetics.[14] The oil of this plant is used to treat acne, eczema, and other skin conditions.

Infusion of its leaves is used to wash children against sickness and decoction of the leaves to combat influenza. The macheted leaves are applied as a poultice to heal wounds. Fresh leaves are used to treat fever.[15,16] However, it has been reforested in Senegal, particularly in the Fatick region, to combat salinization.[17]

Bark and leaves are used in folk medicine as tranquilizers, sedatives, antimalarials, and analgesics.[18]

4. Phytochemical studies and biological activities

The chemical composition of the essential oil has been the subject of a few leaf studies. In Indonesia, the essential oils of *M. leucadendra* leaves were dominated by 1, 8-cineole (44.76%), α-terpineol (5.93%) and limonene (4.45%) [14]. *M. leucadendra* oil from Brazil was also made up of monoterpenes, in which 1,8-cineole was largely predominant (49%), followed by α-terpineol (7.6%) and terpinene-4-ol (4.3%).[19] In Senegal, the essential oil of *Melaleuca leucadendra* leaves harvested in the Fatick region was dominated by methyleugenol [20]. A very recent study (2024)[21] showed three main constituents of *Melaleuca leucadendra* essential oil, namely 1,8-cineole (61.46%), α-terpineol (7.87%) and d-limonene (6.23%). The effect of harvesting seasons, storage period, and distillation time was studied on the oil composition of *Melaleuca leucadendra (L)* growing in India.[22] The results showed that the essential oils comprised mainly oxygenated sesquiterpenes (> 88%), followed by sesquiterpene hydrocarbons and monoterpenoids. The major constituent reported is (E)-nerolidol (⩾ 90%). The study carried out by Bautista-Silva et al. (2020)[23] demonstrated that the essential oil derived from *Melaleuca leucadendra* was mainly composed of monoterpenoids (77.43%). In addition, the analysis revealed four major compounds: α-pinene (9.06%), limonene (32%), 1,8-cineole (17.32%), and viridifora (14.89%). Another study (2024)[24] carried out on *Melaleuca leucadendra* from Brazil showed that the essential oil obtained is composed of monoterpenes (43.76%), the main compounds of which are α-pinene (8.19%), β-terpineol (17.09%) and α-terpineol (6.65%).

Figure 2: Tree, leaves and flowers of *Melaleuca leucadendra (L)L.* Photo by M. Mbodji January 2023 in Dakar

The evaluation of biological activities has been the subject of several studies. Farag et al. have shown that *M. leucadendra* essential oil possesses antiviral, antimicrobial, and antioxidant activities.[25] In Pakistan (2020), Sadique et al carried out an in vitro antimicrobial study using diffusion and microdilution on agar wells, and the oils tested showed bacteriostatic and bactericidal effects against food-borne pathogens tested at 4-8 µg/mL. The temporal assay showed a bactericidal effect of the oil for four weeks.[26] In Indonesia (2021), a study was carried out on the larvicidal activity of *Melaleuca leucadendra* leaf extracts against *Aedes aegypti*.[27]

In Senegal, a study showed that *Melaleuca leucadendra* essential oils have insecticidal activity.[28] In Indonesia (2024), Zaki et al studied the antibacterial effects of *Melaleuca leucadendra* ecoenzyme on pseudomonas aeruginosa.[29] Péricles Tavares et al. (2023) carried out a phytochemical and antimicrobial evaluation of the hydroalcoholic extract of *Melaleuca leucadendra* leaves on bacteria responsible for bovine mastitis. Results showed the presence of terpenes, glycosylated flavonoids, aglycone flavonoids, terpenes, and tannins, and spectrometry identified 13 chemical superclasses with 88 compounds.

The extract showed antimicrobial activity against Staphylococcus aureus strains from bovine mastitis, demonstrating that it is a promising option in the treatment of the disease. [30] The results of a study carried out on the antibacterial tests of *Melaleuca leucadendra* extract showed strong activity in response to the inhibition of pneumonia-causing bacteria.[31]

Other pharmacological effects have been reported, including antioxidant, anti-inflammatory[32], and antimicrobial activities. [33, 25] Lucas Resende et al. (2024) [24] conducted studies on the essential oil's in vitro photoprotective, antioxidant, and anti-melanoma activities.

Melaleuca leucadendra (L) L., with its many therapeutic virtues, requires more in-depth phytochemical studies in order to characterize its biological activities by researching and detecting the various classes of secondary metabolites found in this species.

5. Materials and methods

5.1. Preparation of plant material

Melaleuca leucadendra (L) L. leaves (figure 3) were collected from the Hann botanical garden (Dakar). They were dried in a dark place for two (2) weeks.

Figure 3: The leaves of *Melaleuca leucadendra (L) L.* Photo by M. Mbodji January 2023 in Dakar

5.2. Extraction method

- Hydrodistillation using a Clevenger-type system

For each extraction, we filled the cylindrical flask with a specific mass of plant material (55-70 g). The 2-liter flask, 2/3 full of water, was brought to the boil by a heating mantle. Under the effect of heat, the odorant molecules contained in the plant's secretory glands are released in the form of an azeotropic mixture. Although most constituents have boiling temperatures in excess of 100 °C, they are mechanically entrained with water vapor. Condensation cooling leads to water-essential oil separation by decantation. The “Clevenger” system, recommended by the European Pharmacopoeia, enables the aqueous distillate phase to be recycled to the boiler via cohobage. In this way, water and poultry molecules are separated into an aqueous phase (hydrolat) and a supernatant organic phase (essential oil) by their differences in density in the essencier. After 3 hours of extraction, the essential oil is weighed and stored in glass bottles.

- Extraction by maceration

We used distilled water, ethanol/water (50:50, v/v), and methanol as extraction solvents. For this purpose, 20 g of *Melaleuca leucadendra (L) L.* leaf powder was weighed for each extraction using 200 mL solvent, except for the hydro-ethanol extraction, which was prepared in 50/50 proportions (100 mL distilled water and 100 mL ethanol). Extractions were carried out under agitation for 24 hours. After filtration, the filtrates were placed in a ventilated oven for evaporation.

- Phytochemical screening

After extraction, we turned our attention to the phytochemical screening of the extracts. These tests are based on staining and precipitation reactions to characterize the different families of secondary metabolites in the extracts.[34]

5.3. Dosages

- Determination of extract polyphenols

The phenolic compound content of extracts was determined using the Folin-Ciocalteu reagent[7], according to an assay method written by Muller et al. in 2010. The reagent, which consists of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), is reduced during phenol oxidation. The blue color produced is proportional to the total polyphenol content.

To achieve this, a 0.1 mL volume of extract was mixed with 2 mL of freshly prepared 2% sodium carbonate (Na_2CO_3) solution and vortexed. After five minutes, 100 µL of Folin-Ciocalteu reagent was added to the mixture. A gallic acid range was prepared in parallel. After 30 minutes of incubation in the dark, absorbance is read at 760 nm using a UV-visible spectrophotometer. The quantity of polyphenol is deduced from the equation of the calibration line and expressed in milligrams of gallic acid equivalent per gram of extract (mg EAG/g extract).

- Determination of flavonoids in extracts

The determination of flavonoids in extracts is based on the formation of a complex between aluminum trichloride ($AlCl_3$) and flavonoids.[35]

The aluminum trichloride ($AlCl_3$) method is used for flavonoid quantification. A 2 mL volume of extract was mixed with 2 mL of a 2% aluminum chloride ($AlCl_3$) solution. In parallel, a quercetin range was prepared. Absorbance was read at 430 nm. The quantity of flavonoids is deduced from the equation of the calibration line established with quercetin. Results are expressed as milligram quercetin equivalent per gram extract (mg EQ/g extract).

- Determination of hydrolyzable tannins in extracts

Determination of hydrolyzable tannins was performed using the ferric chloride method reported by Essis et al. in 2003.[36]

A 3.5 mL volume of ferric chloride (0.01 M $FeCl_3$ in 0.001 M HCl) was added to 1 mL extract. After homogenization, absorbance was read at 660 nm. The hydrolyzable tannin content was determined by reference to a calibration curve obtained with tannic acid. Results are expressed in milligrams of tannic acid per gram of extract (mg EAT/g extract).

- Determination of essential oil polyphenols

The determination of polyphenols is carried out using the Folin-Ciocalteu reagent.

To do this, a 0.1 mL volume of the essential oil extract at a concentration of 1 mg/mL (50 mg of essential oil was solubilized in 50 mL of DMSO) was mixed with 2 mL of a freshly prepared 2% sodium carbonate (Na_2CO_3) solution, and the whole was vortexed. After five minutes, 100 µL of Folin-Ciocalteu reagent (1 N) was added to the mixture, which was left to incubate for 30 minutes in the dark. The absorbance was read at 700 nm. The results were expressed as milligram equivalents of gallic acid per gram of essential oil extract (mg GAE/g EO).

- Determination of flavonoids in the essential oil

A volume of 2 mL of essential oil extract was mixed with 2 mL of a 2% aluminum chloride ($AlCl_3$) solution. In parallel, a quercetin range was prepared. Absorbance was read at 430 nm. The flavonoid content was deduced from the equation for the quercetin calibration line. It is expressed in milligram equivalents of quercetin per gram of essential oil extract (mg EQ/g of EO).

5.4. Determination of antioxidant activity by the DPPH method

This method's principle is reducing violet DPPH (2,2-diphenyl-1-picrylhydrazyl) to yellow 2,2 diphenyl-1-picrylhydrazine. DPPH- absorbs at 517 nm, but when reduced by an antioxidant, its absorption decreases. [37]

- Determination of the antioxidant capacity of the essential oil extract

To determine the antioxidant capacity, 20 µL of the essential oil extract was taken and made up to 200 µL with ethanol. Next, 3.8 mL of the 0.1014 mM DPPH solution was added to the diluted extracts. A trolox range was prepared in parallel. After 30 minutes of incubation in the dark, the absorbance was read at 517 nm.

- Determination of the antioxidant capacity of the three extracts

To determine the antioxidant capacity, a volume of 20 µL of each extract was taken and made up to 200 µL with the extraction solvent. Next, 3.8 mL of the 0.1014 mM DPHH solution was added to the diluted extracts. A trolox range was prepared in parallel. After 30 minutes of incubation in the dark, the absorbance was read at 517 nm.

6. Results and discussion

6.1. Oil extraction yield

The yield of essential oils was calculated as the ratio between the mass of essential oil and the mass

of raw plant material used. The result is expressed as a percentage. The yield (in %) is presented in the following table:

Table II: Yield results

Mass of plant matter (g)	Mass of essential oil (g)	Yield in %
800	14.0143	1.75

Table 2 shows that a yield of 1.75% was obtained after extraction of the essential oil.

- Yield of extraction by maceration

To calculate the extraction yield, it is first necessary to determine the mass of the macerates. The mass of each macerate is determined by the difference between the mass of the tube containing the extract and the mass of the empty tube. Once these different masses have been obtained, the yield is determined. The yield of each extract is obtained by dividing the dry extract (macerate) by the mass of the plant material (leaf powder), multiplied by one hundred (100).

Table III: Yields of extracts

Extracts	Yield in %
Aqueous extract	5.61
Hydroethanol extract	4.89
Methanolic extract	5.97

The results show that of the three extracts, the methanolic extract had the highest yield (5.97%), followed by the aqueous extract with a yield of 5.61%. The hydroethanol extract had the lowest yield (4.89%). The different yields may reflect the nature of the compounds present in the raw material.

6.2. Phytochemical screening

The aim was to detect the different families of secondary metabolites in *Melaleuca leucadendra (L) L* leaves. These tests were carried out on the different extracts (aqueous, methanolic, and hydro-ethanolic).

Phytochemical analysis of the three extracts of *Melaleuca leucadendra (L) L*. leaves show a relative richness in secondary metabolites. The aqueous extract reveals the presence of polyphenols, flavonoids, condensed and gallic tannins, coumarins, and alkaloids. The presence of terpenes and the absence of saponins were observed. The methanolic extract revealed the presence of polyphenols, flavonoids, gallic tannins, coumarins, and alkaloids. The presence of terpenes and condensed tannins and the absence of saponins were noted. The hydroethanol extract revealed the presence of polyphenols, flavonoids, condensed and gall tannins, coumarins, and alkaloids. There was a slight presence of terpenes and saponins.

Table IV: Results of phytochemical screening of the three extracts

Compounds	**Aqueous extract**	**Methanolic extract**	**Hydroethanol extract**
Terpenes	+	+	+
Polyphenols	+++	+++	+++
Flavonoids	+++	++	+++
Saponins	-	-	+
Condensed tannins	+	+	+
Gallic tannins	+	+	+++
Coumarins	+++	++	+++
Alkaloids	+++	+++	+++

Legend: *(+): Presence, (-): absence*

6.3. Dosages

- Determination of polyphenols

The polyphenol content is determined using the Folin-Ciocalteu reagent. Gallic acid is used as a standard, and the result is shown in the calibration curve below.

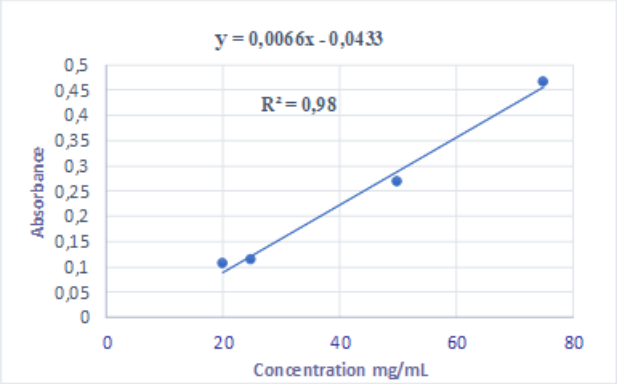

Figure 4: Gallic acid calibration curve

Table V: Polyphenol content of extracts

Extracts	**Polyphenol content in mg EAG/g extract**
Aqueous	86.48 ± 0.075
Hydroethanolic	102.39 ± 0.378
Methanolic	110.72 ± 0,075
Essential oil	21, 788 ± 0,075

From the results found, we noted that the polyphenol content of the methanolic extract is the highest with content of 110.72 mg EAG/g extract, followed by the hydro-ethanolic extract, and the aqueous extract respectively, 102.39±0.38 mg EAG/g extract and 86.48 mg EAG/g extract. The essential oil had the lowest polyphenol content at 21.788 mg EAG/g extract.

- Flavonoid assay

The determination of flavonoids in extracts is based on the formation of a complex between $AlCl_3$ and flavonoids. Absorbance is read at 430 nm.

Quercetin was used as the standard, the calibration curve having the equation below.

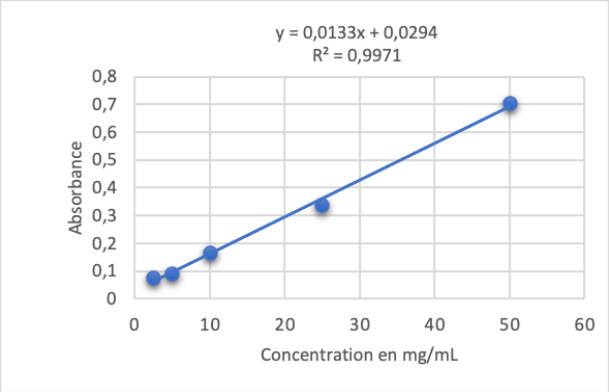

Figure 5: Quercetin curve

Table VI: Flavonoid content of extracts

Extracts	Flavonoid content (mg EQ/g extract)
Aqueous	61.323 ± 0.827
Hydroethanolic	33.203 ± 0.300
Methanolic	100,285 ± 1,578
Essential oil	93,097 ± 0,827

Quantification of flavonoid content revealed that the methanolic extract contained the highest flavonoid content at 100.285 mg EQ/g extract, followed by the essential oil extract at 93.097 mg EQ/g extract. The hydroethanol extract had the lowest content at 33.203 mg EQ/g extract.

- Determination of hydrolyzable tannins

The content of hydrolyzable tannins was determined using the ferric chloride method reported by Essis et al. Tannic acid was used as the standard, the calibration curve having the equation below.

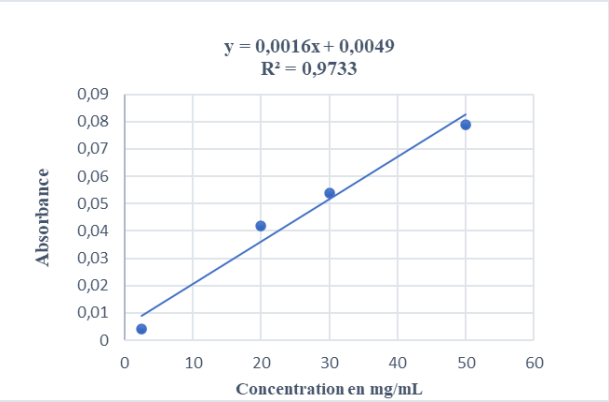

Figure 6: Tannic acid curve

Table VII: Hydrolysable tannin content

Extracts	Hydrolysable tannin content (mg EAT/g extract)
Aqueous	162.250 ± 3.437
Hydroethanolic	106.000 ± 0.312
Methanolic	147,250 ± 2,812

The results show that the aqueous extract contains the highest content of hydrolyzable tannins with content of 162.250 mg EAT/g extract, followed respectively by the methanolic extract and the hydro-ethanolic extract with contents of 147.250 mg EAT/g extract and 106 mg EAT/g extract respectively.

6.4. Determination of antioxidant activity

- Antioxidant capacity of the essential oil

The antioxidant capacity is deduced from the trolox calibration line, the equation of which is given below.

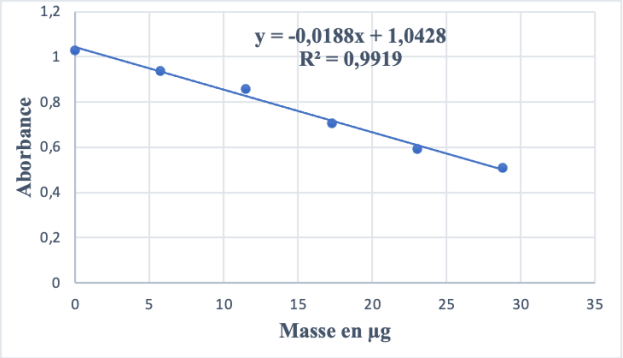

Figure 7: TROLOX calibration curve

Antioxidant capacity is expressed in milligrams TROLOX equivalent per gram of essential oil (mg ETr/ g EH).

Table VIII: Antioxidant capacity of essential oils

Extract	Antioxidant capacity (mg ETr/g EO)
Essential oil	0.347 ± 0.005

The result shows that our essential oil extract contains an antioxidant capacity of 0.347 mg ETr/ g EO.

- Determination of the IC50 of the essential oil

Having determined the antioxidant capacity, the evolution of antioxidant activity at different concentrations of the essential oil was evaluated. Figure 8 shows that our essential oil extract is capable of inhibiting at least 50% of the DPPH-induced radical activity. Figure 8 shows that our essential oil extract has an IC50 of 0.15 mg/mL.

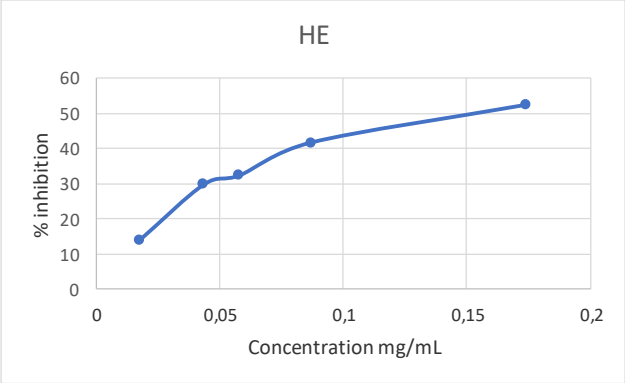

Figure 8: Curve of % inhibition as a function of different concentrations of EO

- Antioxidant capacity of extracts

The antioxidant capacity is deduced from the TROLOX calibration line (figure 9).

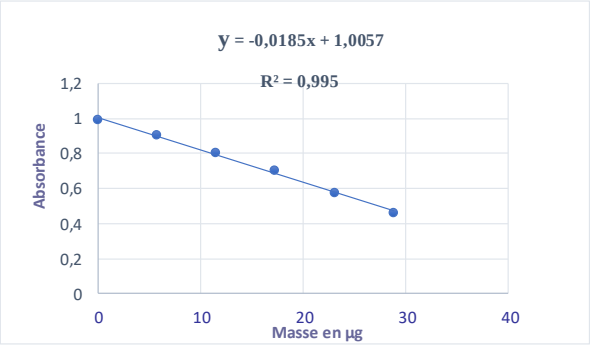

Figure 9: TROLOX calibration curve

Antioxidant capacity is expressed in milligram TROLOX equivalent per gram of dry matter (mg ETr/g Ms).

Table IX: Antioxidant capacity results

Extracts	Antioxidant capacity (mg ETr/g Ms)
Aqueous	54,543 ± 0,450
Methanolic	64,224 ± 0,240
Hydroethanolic	133,144 ± 0,960

The results show that the hydro-ethanolic extract has the highest antioxidant capacity of the three, with 133.144 mg ETr/g Ms, followed by the methanolic extract with 64.224 mg ETr/g Ms and finally the aqueous extract with 54.543 mg ETr/g Ms.

As well as simply ranking the extracts in order of antioxidant capacity, it is important to note that the difference in capacity may be due to the polarity of the solvents used for extraction.

Hydro-ethanol extracts are often more efficient at extracting a wide range of compounds, including antioxidants, because water and ethanol have different polarities that allow them to solubilise a wide variety of chemical compounds. Thus, the hydroethanol extract may contain a greater diversity of antioxidants than the aqueous or methanol extracts. This suggests that the choice of solvent can have a significant impact on the composition and antioxidant capacity of the extract obtained.

- Determination of extract IC50

Having determined the antioxidant capacity, the evolution of antioxidant activity at different concentrations of the extracts was evaluated. The results (fig 11, fig 12 and fig 13) show that all the extracts are capable of inhibiting at least 50% of the free radical activity. A lower IC50 value means greater antioxidant efficiency.

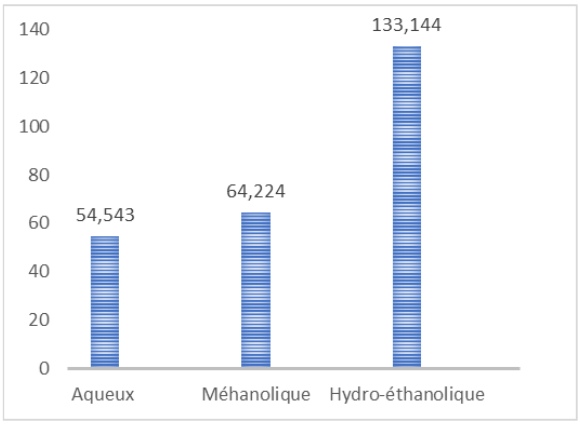

Figure 10: Histogram of antioxidant capacities of the three extracts

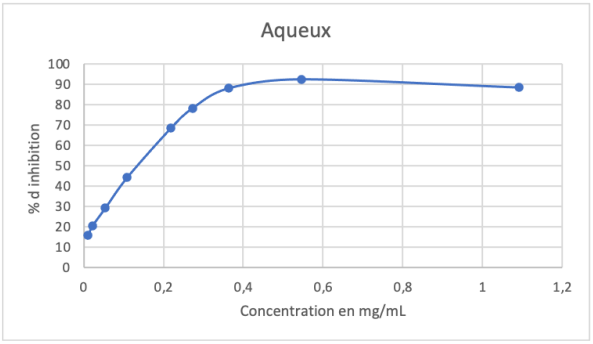

Figure 11: Changes in antioxidant activity at different concentrations of aqueous extract

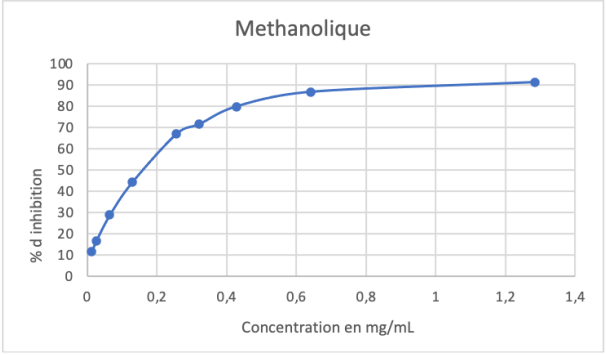

Figure 12: Changes in antioxidant activity at different concentrations of methanol extract

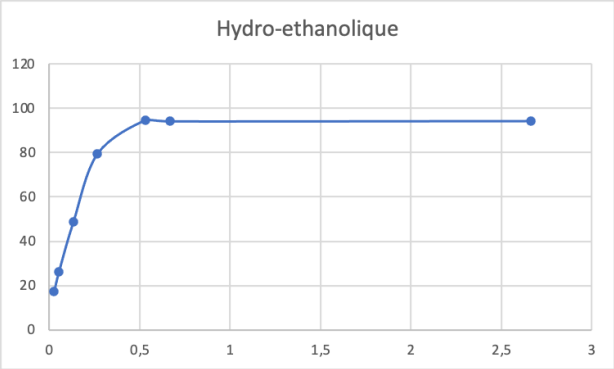

Figure 13: Changes in antioxidant activity at different concentrations of hydroethanol extract

According to the figures (fig11 fig12 and fig13), antioxidant activity is concentration dependent. So, each time the concentration of the extracts increases, the percentage of inhibition of the DPHH- free radical also increases.

Both the aqueous and methanolic extracts showed very high levels of activity. At a concentration of 0.545 mg/mL, the former achieved a percentage inhibition of 92.50% and the latter 91.29% at a concentration of 1.28 mg/mL. We can say that antioxidant activity depends on the dose and the nature of the compounds contained in the extract. We will then determine the concentration resulting in 50% inhibition (IC50) of each extract, the results of which are given in Table 10.

Table X : IC50 of extracts

Extracts	**IC50 (mg/mL)**
Aqueous	0,123
Methanolic	0,156
Hydroethanolic	0,142

According to the IC50 results, the aqueous extract had the lowest IC50 value (0.123 mg/mL), indicating that it had the highest antioxidant activity of the three extracts. This suggests that the active compounds present in this extract are highly effective at neutralising free radicals. The hydroethanol extract had a slightly higher IC50 value (0.142 mg/mL) than the aqueous extract. The methanolic extract has the highest value, meaning that it has the lowest antioxidant activity of the three. The methanol-soluble compounds appear to be less effective for antioxidant activity compared with those in the other extracts.

The difference in IC50 values can be attributed to the nature of the antioxidant compounds extracted by each solvent. Water and hydroethanol mixtures are often effective in extracting phenolic compounds, flavonoids and other water-soluble antioxidants, which are generally potent. The lower efficiency of methanolic extracts could be due to the lower affinity of methanol for certain antioxidant compounds, which is less efficient than those extracted by water.

7. Conclusion

The use of medicinal plants in phytotherapy has received great interest in the search for biologically active molecules of natural origin. In the present work, we were interested in the phytochemical screening, the determination of polyphenols and flavonoids and the evaluation of the antioxidant activity of our two different extracts from the leaves of the plant *Melaleuca leucadendra (L) L*. From the phytochemical point of view, we carried out the solid-liquid extraction by maceration and the extraction of the essential oil of this plant. Phytochemical screening showed that our plant is rich in secondary metabolites in the three extracts (aqueous, methanolic and hydroethanolic).

Tests on the antioxidant activity of the four extracts, i.e. the extracts obtained by maceration and the essential oil extract, showed that all four extracts have significant antioxidant activity. However, it should be noted that the aqueous extract showed the best antioxidant activity with an IC50 of 0.123 mg/mL.

These results are encouraging and demonstrate the richness of this plant, which is known for its therapeutic properties. Since the leaf extracts from our plant have a very high antioxidant potential, further research is needed to identify the compounds responsible for the antioxidant activity.

Other activities such as antibacterial, anti-inflammatory and antifungal activity could also be evaluated.

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