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Phytochemical Composition, Antioxidant and Antibacterial Activities of Extracts from Different Parts of *Brocchia cinerea* (Vis.)

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Abstract: *Brocchia cinerea* (Vis.) is an Asteraceae-family plant widely used in traditional medicine in Southeastern Morocco to treat several ailments. The aqueous extracts of flowers, leaves, stems and aerial parts of *B. cinerea* were subjected to a preliminary phytochemical screening test for various constituents. The polyphenols, flavonoids, and tannins contents were determined. Moreover, the antioxidant activity was evaluated by DPPH scavenging assay and ferric reduction antioxidant power. However, the antibacterial activity was evaluated against six pathogenic bacteria. Phytochemical screening of different parts of *B. cinerea* revealed alkaloids, flavonoids, saponins, tannins, and terpenoids. The leaves extract showed the highest polyphenols concentration (27.15±0.92 mg GAE/g of Ext) and flavonoids (17.46±0.66 mg QE/g of Ext), while flowers extract was highest in tannin (9.57±1.24 mg TAE/g of Extract). All extracts of *B. cinerea* showed moderate to high antioxidant power, among which the leaves extract demonstrated the strongest antioxidant activity, with an IC50 value of 0.99 mg/mL and an optical density of 1.15. In the case of antibacterial activity screening, leaves extract showed the highest inhibition zone diameters ranging from 9 to 27 mm against the tested bacteria. The results demonstrate that the aqueous extracts of different parts of *B. cinerea*, especially the leaves, could be developed as pharmaceutical products.

Keywords: *Brocchia cinerea*; *Cotula cinerea*; extract; antioxidant activity; antibacterial activity.

1. Introduction

Continuous exposure to different aggressors causes an increase in the production of reactive species and cellular deterioration and, in the long run, contributes to aging and other organic disorders [1]. Oxygen, nitrogen, and sulfur reactive species, including free radicals, trigger the depletion of antioxidants in the immune system, alter the expression of genes, and generate abnormal proteins [2].

The oxidation mechanism is one of the most critical pathways for generating free radicals in food, drugs, and even living systems [3]. The human body has an inherent

antioxidative mechanism to prevent free radicals from attacking biological cells by stabilizing or deactivating them [4]. Due to the depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary [5,6].

Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinone, and gallic acid esters have been suspected of causing negative health effects, as well as they also show moderate antioxidant activity and low solubility [7]. Recently, there is an increasing interest in finding natural antioxidants from plant materials to replace synthetic ones.

Plants contain a large variety of substances that exhibit an ability to prevent the destructive process caused by oxidative stress, such as vitamin E, vitamin C, carotenes, xanthophylls, tannins, and phenolics [8]. These natural antioxidants have been shown to prevent lipid peroxidation (by inactivating lipoxygenase), scavenge free radicals and active oxygen species via a reaction cycle, and chelate heavy metal ions [2].

Edible and non-edible plants contain phenolic compounds, which have many biological effects, including antibacterial activity. Microorganisms are causative factors for various diseases as well as for the spoilage and deterioration of food, pharmaceutical, and cosmetic products. Furthermore, the number of bacteria that resist current antibiotics has dramatically increased in recent years [9], indicating the need for new antibacterial agents. Moreover, concerns about the toxicity and carcinogenicity of synthetic antibacterial agents [10] have heightened the quest for natural alternatives.

The use of medicinal plants for prevention or treatment is an old tradition, but recently, it has sparked an increasing interest by researchers studying their biological properties [11]. Natural substances contribute to the detoxification and elimination of reactive species [12] and the discovery of novel antibacterial compounds [13]. Brocchia cinerea (Vis.), locally known as Al gartoufa, is one of those matrices containing many biomolecules, including phenolic compounds [13].

Brocchia cinerea (Anthemideae tribe) is a small annual herb with discoid capitula and homogamous hermaphrodite florets. It is extensively distributed in the Sahara desert and represents one of the monotypic Anthemideae genera, characterizing the North African flora [14].

It has been used, since ancient times, both for medicinal purposes and livestock nutrition. B. cinerea is traditionally used as a decoction and infusion to treat colic, diarrhea, digestive troubles, headaches, fever, migraines, coughing, broncho-pulmonary cooling, rheumatoid arthritis, inflammation, urinary and pulmonary infections [15,16].

The phytochemical screening of *B. cinerea* revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, and cardenolides [17]. These compounds have a broad biological effect, including antibacterial and antioxidant proprieties, which may justify the traditional use of this plant [15].

This present study investigates the content of polyphenols, flavonoids, and tannins of aqueous extracts from leaves, flowers, stems, and aerial parts (whole plant) of *B. cinerea* and evaluates its antioxidant effect in comparison with ascorbic acid (commercial standard antioxidant). The antibacterial activity was also studied against pathogenic bacteria strains.

2. Materials and Methods

2.1. Plant material.

Brocchia cinerea plant was collected during the flowering period in March 2020 from the H'ssia of Alnif region (Southeastern Morocco). A Voucher specimen (RAB 110972) was deposited in the National Herbarium of the Scientific Institute Botany Department (Rabat, Morocco).

The harvest was divided. The aerial parts of *B. cinerea* were washed, sorted, and dried at room temperature away from light and humidity for 15 days. Leaves, flowers, and stems were separated manually, constituting individual fractions beside the whole aerial part. Afterward, each part was finely pulverized using a mill blade and conserved until the analysis time.

2.2. Extraction.

The four different fractions of *B. cinerea* (35 g) were extracted in soxhlet with water using the technique of Netien *et al*. [18]. After that, all extracts were concentrated in a rotary evaporator under a vacuum to get crude extracts, which are subsequently dried and stored in a desiccator until use.

2.3. Phytochemical screening.

Preliminary phytochemical testing for the presence of various compounds was conducted, using the following standard methods: Trease *et al*. [19] for Tannins, Sofowora [20] for Cadiac glycosides, Gibbs [21] for Steriods, Harborne [22] for Alkaloids, Kumar *et al*. [23] for Saponins, Salkowski test for Terpenoids [24], and Khandelwal [25] for compounds like Flavonoids compounds, and mucilages.

2.4. Total phenolic contents.

The contents of phenolic compounds in extracts were determined according to Orthofer and Lamuelas-Raventos [26], using the Folin-Ciocalteu colorimetric method, and Gallic acid (GA) was used as the standard phenolic compound.

All the measurements were taken in triplicate, and mean, and standard deviation values were calculated.

2.5. Total flavonoid determination.

The amount of total flavonoids in *B. cinerea* extracts was determined by the colorimetric method described previously by Park *et al*. [27]. All measurements were performed in triplicate.

2.6. Tannin determination.

Extracts of tannin contents were determined by the method of vanillin described by Julkimen-Titto [28].

2.7. Antioxidant activity.

2.7.1. DPPH radical scavenging activity.

The DPPH assay was carried out as described by Kazemi [29] with some modifications. Briefly, 2 mL of extract solution tested at different concentrations (2.5 – 0.16 mg/mL) were added to 1.0 mL of 0.08 mg/mL freshly made ethanolic solution of DPPH radical (DPPH.). The contents were vigorously mixed, incubated for 30 min in the dark at room temperature, and the absorbance was measured at 517 nm. Ascorbic acid (vitamin C) was used as a positive control. This test was done in triplicate. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using the following equation [30]:

$$\text{Percent inhibition of DPPH activity (I\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where $A_{control}$ and A_{sample} are the absorbance values of the control and the test sample, respectively, a percent inhibition versus concentration curve was plotted, and the concentration of sample required for 50% inhibition was determined and represented as IC50 value for each of the test solutions.

2.7.2. Ferric Reducing Antioxidant Power (FRAP)

Reductive ability was investigated by the Fe^{+3} to Fe^{+2} transformations in the presence of extracts, using the method of Oyaizu [31]. The reducing power tests were run in triplicate.

2.8. Antibacterial activity.

2.8.1. Microorganisms.

Gram-positive bacteria, including *Staphylococcus aureus* (*S. aureus*) and *Streptococcus faecalis* (*S. faecalis*) *and Gram-negative including Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. Pneumoniae*), *Salmonella Kentucky* (*S. Kentucky*), *and Pseudomonas aeruginosa* (*P. aeruginosa*) were used for the antibacterial study. All the stock cultures were collected from Mohamed V regional hospital of Meknes (Morocco). All bacterial strains were grown and maintained on Muller Hinton agar (Biokar, Beauvais, France) at 37°C. The bacteria were subcultured overnight in Muller Hinton broth (Biokar, Beauvais, France), which was further adjusted to obtain turbidity comparable to McFarland (0.5) standard.

2.8.2. Disc diffusion assay.

The disc diffusion assay for antibacterial susceptibility testing was carried out according to the standard method of Chebaibi *et al*. [32] to assess the presence of antibacterial activities of the *B. cinerea* extracts. Briefly, bacterial suspensions were inoculated onto Mueller Hinton agar by the swabbing method. Sterile 6-mm-diameter paper discs were soaked with 20µl of extracts to be tested and deposited on each agar-medium plate.

The 10-µg Gentamicin standard antibiotic disc was used as a positive control for *S. aureus*, *K. pneumonia*, *S. faecalis*, *E. coli*, and *S. Kentucky*, while the 10 µg Imipenem disc was used for *P. aeruginosa*. The negative control was Dimethylsulfoxyde [DMSO] (Sigma-Aldrich, Buchs, Switzerland). After incubation at 37 °C for 24 hours, the inhibition zones around each disc were measured in mm. The tests were done in triplicate.

2.8.3. Microdilution assay.

The assay was performed on 96-well microplates as described by Chebaibi *et al*. [32]. Briefly, decreasing concentrations of plant extracts were prepared in DMSO, then 20 µl of bacterial suspensions in a suitable growth medium (0.5 Mac Farland) and 160 µl of Mueller Hinton Broth were added to each well of a sterile 96-well microtitre plate.

Positive controls were wells with growth medium, bacterial suspension, and negative controls were wells with growth medium and plant extracts. At 37°C, the microplates were incubated for 24 hours.

Minimum Inhibitory Concentration (MIC) was evaluated, after the incubation, by the addition of 40 µl 2,3,5-diphenyltetrazolium chloride [TTC] (0.2 mg/ml), which reveals the bacterial growth by the appearance of a reddish color. However, the Minimal Bactericidal Concentration (MBC) was evaluated by streaking 5 µl of the wells that did not show any bacterial growth on Mueller Hinton agar plates and incubated at 37°C for 24 hours.

MIC is the lowest concentration of plant extracts that does not show bacterial growth. When 99.9% of the bacterial population is killed at the lowest concentration of plant extracts, it is termed MBC.

Moreover, the plant extracts can be classified as bactericidal if the ratio MBC/MIC is inferior or equal to 4 and as bacteriostatic if the ratio MBC/MIC is superior to 4 [32].

2.9. Statistical analysis.

Statistical analysis was done using XLSTAT (2019) version 4.2. One-way ANOVA and Tukey's test (HSD) were conducted. The level of significance was determined at $p< 0.05$. Results were presented as mean values ± SD (standard deviations).

3. Results and Discussion

3.1. Phytochemical screening.

The results and observations of phytochemical screening are summarized in Table 1.

Table 1. Phytochemical screening of *B. cinerea* extracts.

Phytochemical	Observed changes	Results			
		AP	Lv	Fl	St
Tannins	Greenish color	+	+	+	+
Alkaloids	Creamy white precipitate	+	+	+	-
Flavonoids	Red color	+	+	+	+
Terpenoids	Red color	+	+	+	-
Steroids	Greenish color	+	+	-	+
Cardiac glycosides	Reddish-brown color	-	-	-	-
Saponins	Formation of stable foam	+	+	-	-
Mucilages	Formation of a fluffy precipitate	+	+	-	+

(+): Presence of phytochemicals, (-): Absence of phytochemical, AP: aerial part, Lv: leaves, Fl: flowers, St: stems.

The phytochemical screening showed that all the tested phytochemicals were present in leaves and aerial parts extracts of *B. cinerea* except cardiac glycosides. The extract of stems contained tannins, flavonoids, steroids, and mucilages but was devoid of alkaloids, cardiac

glycosides, terpenoids, and saponins, whereas flowers extract contained only four phytochemicals: tannins, alkaloids, flavonoids, and terpenoids.

This result showed that *B. cinerea* leaves contain more phytochemicals than other plant organs. The richness in these secondary metabolites may be responsible for the therapeutic effects observed in *B. cinerea* as anti-inflammatory, analgesic, antiseptic, and treating stomachaches [33].

The results relating to the presence or absence of secondary compounds are similar to those found by Djellouli *et al*. [17] and Mabroka *et al*. [34].

3.2. Phenolic, flavonoid, and tannin contents.

It is well known that polyphenols, flavonoids, and tannins are the important antioxidant substances that are obtained from most natural plants [35,36]. Phenolic compounds are a class of antioxidant agents that act as free radical terminators [37,38]. The mechanisms of action of flavonoids are through the scavenging or chelating process [39]. Hydrolyzable tannins, having galloyl groups, exhibit stronger antioxidant effects [40,41]. The different parts of *B. cinerea* were assayed for polyphenols, flavonoids, and tannins, and the obtained results are given in Table 2.

Table 2. Polyphenols, flavonoids, and tannins contents of different parts of *B. cinerea* (mean±SD).

Extract	Total phenolic contents (mg GAE/g of Extract)	Flavonoids (mg QE /g of Extract)	Tannins (mg TAE /g of Extract)
AP	23.03 ± 1.08^{bc}	11.69 ± 0.92^{ab}	6.41 ± 1.37^{ab}
Lv	27.15 ± 0.92^{c}	17.46 ± 0.66^{b}	9.57 ± 1.24^{c}
Fl	17.69 ± 1.14^{b}	13.81 ± 1.17^{b}	7.12 ± 0.75^{b}
St	8.40 ± 0.71^{a}	5.33 ± 0.86^{a}	2.94 ± 0.93^{a}

Means within a column, lacking a common superscript, differ ($p<0.05$). Fl: Flower extract, Lv: Leave extract, St: Stem extract, AP: Aerial part extract, GA: Gallic acid, Q: Quercitin, TA: Tannin acid.

The data indicated that the leaves extract showed the highest contents of polyphenol and flavonoid. The polyphenol contents of the different parts of the *B. cinerea* plant measured by Folin Ciocalteu reagent in terms of gallic acid equivalent varied from 8.40±0.71 to 27.15±0.92 mg GAE/g of an extract with a descending order of Lv > AP > Fl > St. The amounts of flavonoid contents, in terms of quercetin equivalent, were ranged from 5.33±0.86 to 17.46±0.66 mg QE/g extract, while the tannin contents in terms of tannic acid equivalent were between 2.94±0.93 and 9.57±1.24 mg TAE /g of an extract with a descending order of Lv > Fl > AP > St. No significant differences ($P<0.05$) were observed among the values of flavonoids in aerial parts, leaves and flowers. However, for total phenolic and tannins, the difference was significant between leaves, flowers, and stems.

These results showed that the polyphenol, flavonoid, and tannin contents have obviously varied from one plant organ to another.

Total phenolics and flavonoids from the aerial parts of *B. cinerea* in the present study were lower than those reported by Kasrati *et al*. [42], who studied the dry aerial part of *B. cinerea* harvested in Zagora (Southern Morocco).

In another study on the flower parts of *B. cinerea* harvested in the Adrar region (Southern Algeria), Belyagoubi-Benhammou *et al*. [43] obtained a total phenolic content of 22.22 ± 0.41 mg gallic acid equivalent and a flavonoid yield of 3.93 ± 0.06 mg quercetin equivalent.

3.3. Antioxidant activity.

3.3.1. DPPH free radical scavenging activity.

To evaluate the scavenging effect of the extracts in this study, DPPH reduction was investigated against ascorbic acid as a positive control. The more antioxidants occur in the extract, and the more DPPH reduction will occur. Figure 1 represents the dose-response curve of DPPH scavenging activities of the extracts studied.

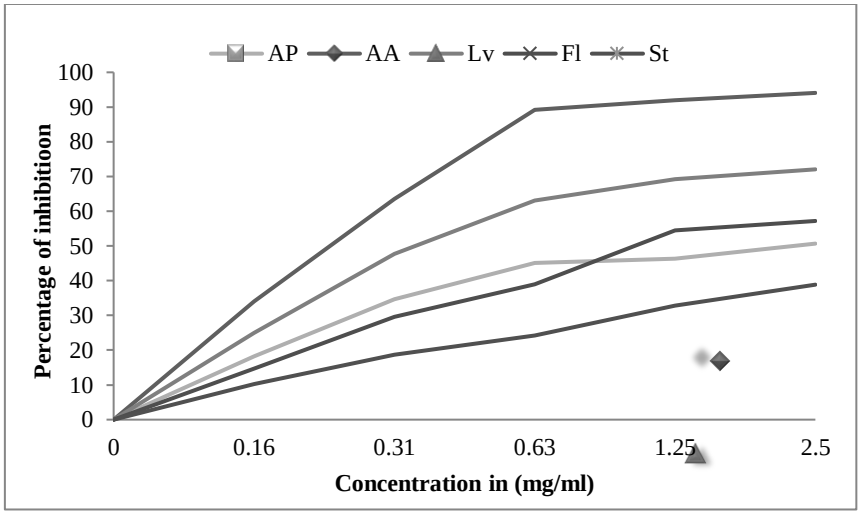

Figure 1. Antioxidant activity of extracts of different parts of *B. cinerea* measured by the DPPH method. AP: aerial parts, Lv: leaves, Fl: flowers, St: stems, AA: Ascorbic acid.

The ANOVA results indicated that there was a significant difference in mean percentage scavenging between all the tested extracts (Leaves, flowers, stems, and aerial parts) at the maximum concentration of 2.5 mg/ml.

The leaves extract was the most active with an inhibition percentage of 72.06± 0.95%. Furthermore, the stems seem to be the least inhibitor ones of DPPH free radicals (38.86± 0.59%) in comparison to flowers and aerial parts with a maximal inhibition percentage of 57.20± 1.19 and 50.68± 1.35%, respectively. Ascorbic acid showed significantly ($P<0.05$) higher activity with a maximal inhibition percentage of 94.08±0.93% compared to these extracts.

The concentrations that led to 50% inhibition or effectiveness (IC50) are given in Figure 2.

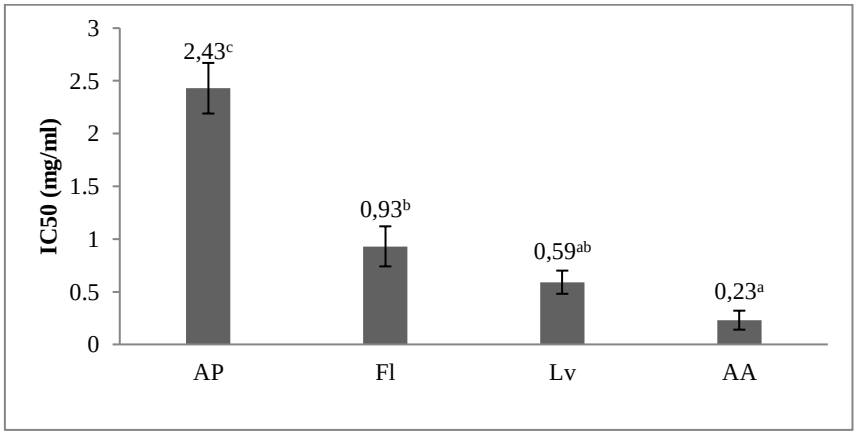

Figure 2. IC_{50} of extracts of different parts of *B. cinerea*. AP: aerial part, Lv: leaves, Fl: flowers, AA: Ascorbic acid. a-c: means lacking a common letter are significantly different ($P < 0.05$).

The highest radical scavenging activity was observed in the leaves extract, whereas the stems extract showed the lowest activity. The IC50 of ascorbic acid was lower than those of all extracts tested ($P<0.05$), which explains its strong radical scavenging activity.

The antioxidant effect observed in these extracts is explained by the presence of compounds that interacted with the free radicals by acting as electron or hydrogen atoms donors.

Numerous studies correlate the antioxidant activity of the plant extracts with the presence of phenolic compounds [44,45,46]. However, other studies came to opposite results [44], and others show very little correlation [48]. In the current study, a positive correlation was also found between total phenolic content and antioxidant activity in all plant extracts ($r= 0.753$, $p<0.05$).

Similar observations were made with the extract of *B. cinerea* aerial parts, collected in southern Algeria, which showed strong antioxidant activity, with an EC50 of 462.19 mg antioxidant/g DPPH and 17.190 ± 1.273 mg of ascorbic acid equivalents/g dry weight (total antioxidant capacity) [43].

Recent studies demonstrated that the interaction of a potent antioxidant with DPPH depends on its structural conformation. The number of reduced DPPH molecules appears to be correlated with the number of available hydroxyl groups [49,50].

The screening of plant extracts, using the DPPH free radical method, proved to be effective for selecting those that could have antioxidant activity [51].

These extracts were rich in radical scavengers, such as phenolics and flavonoids, which intercept the free-radical oxidation chain and donate hydrogen from the phenolic hydroxyl groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids [52].

Besides, tannins have strong antioxidant properties due to a large number of phenolic hydroxyl groups and a high degree of hydroxylation of aromatic rings [53]. Tannins are 15−30 times more efficient in quenching peroxyl radicals than simple phenols [50]. Hydrolyzable tannins, having galloyl groups, exhibited stronger antioxidant effects than flavonoids [54,55]. An increase of galloyl groups, molecular weight, and ortho-hydroxyl structure can enhance their antioxidant activity [56]. While tannins are unlikely to be absorbed by the human body due to their big molecular size, they can still exert antioxidant activity in the digestive tract, protecting lipids, proteins, and carbohydrates from oxidative damage [57].

The leaves extract exhibits a more powerful antioxidant activity than the other extracts and contain more total polyphenols.

These results could be explained by the differences in the distribution of the antioxidant compound in the different plant organs. A similar observation was discovered in *Secondatia floribunda* [58] and *Cassia fistula* [59].

According to Siddhuraju *et al*. [59], the high antioxidant power obtained by the leaves is related to the production of flavonol, proanthocyanidins, anthraquinones, and xanthones. Moreover, Del Bano *et al*. [46] discovered that some antioxidative compounds were selectively biosynthesized by the leaves but not by other organs (flower and stem). This may be because the leaves are under more stress during the photosynthesis process, where the leaf tissues absorb a large amount of light energy.

Photosensitization (the process of transferring absorbed light energy), was produced in chlorophylls, which could trigger the synthesis of highly reactive chemical species at the cellular level [60]. Therefore, free radical scavengers and highly effective antioxidants must be

released by leaf tissues to eliminate ROS (Reactive Oxygen Species) and minimize photosensitization-induced oxidative damage [61].

3.3.2. Ferric Reducing Antioxidant Power (FRAP).

Some investigators reported the antioxidant power to be concurrent with the development of reducing power [62,63].

The FRAP method tests antioxidant activity through the ability of antioxidant compounds to reducing Fe^{3+} ions to Fe^{2+} in the presence of potassium ferricyanide [$K_3Fe(CN)_6$].

The reducing power of aqueous *B. cinerea* extracts is summarized in Figure 3.

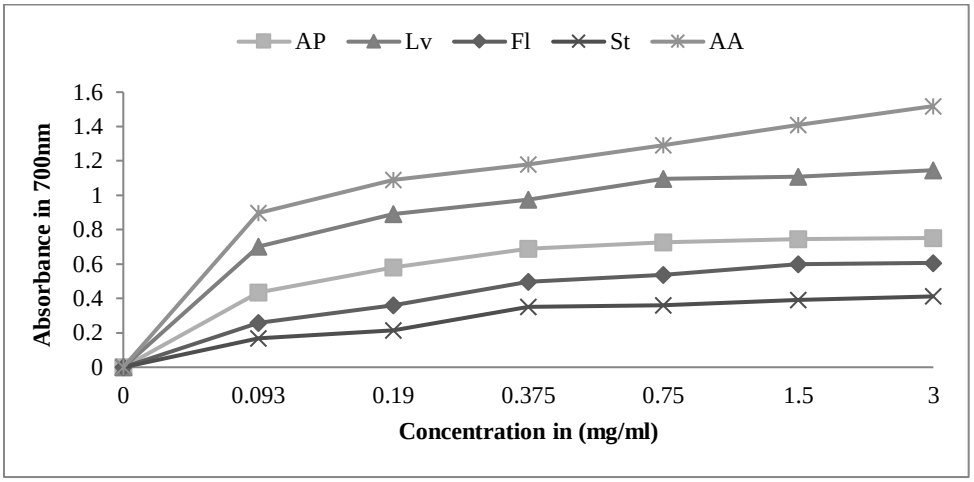

Figure 3. Antioxidant activity of *B. cinerea* extracts by FRAP method. AP: aerial parts, Lv: leaves, Fl: flowers, St: stems, AA: Ascorbic acid.

The optical density (DO) of all extracts studied increases with increasing concentration. At a maximum concentration of 3 mg/ml, the leaves extract exhibited a higher reducing ability (DO= 1.15) than the flowers and aerial parts extracts, which showed a moderate reducing power, with DO values of 0.75 and 0.71, respectively. The lowest DO value was observed in the stem extract (DO= 0.41). Ascorbic acid showed, at all concentrations, a significantly ($P < 0.05$) higher activity compared to the extracts tested.

According to Yen and Chen [64], the extract with a reducing power could act as an electron donor while reducing the oxidized intermediates produced by the lipid peroxidation reaction.

The ability to reduce Fe^{3+} may be attributed to hydrogen donation from phenolic compounds [65,66], which is also related to the presence of a reductant agent [46]. This antioxidant power of various *B. cinerea* extracts and the contents of phenolics and flavonoids exhibited a significant correlation. Similarly, the molecular antioxidant response of phenolic compounds varies remarkably, depending on their chemical structure [67,68] and the position of their hydroxyl group [67,69]. Besides, other chemical components in the extract, such as sugars or ascorbic acid, may cause interference [70].

3.4. Antibacterial activity.

3.4.1. Antibacterial test with the paper disc diffusion method.

The antibacterial activity of *B. cinerea* extracts was determined against six pathogenic bacteria strains, as shown in Figure 4.

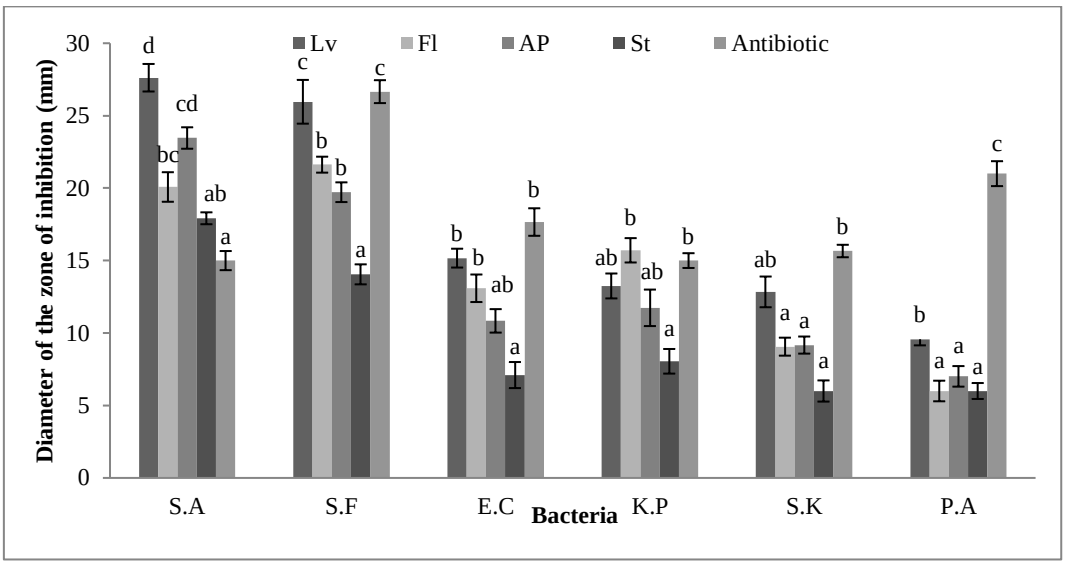

Figure 4. Antibacterial activity of extracts from different parts of *B. cinerea*. AP: aerial parts, Lv: leaves, Fl: flowers, St: stems, E.C.: *Escherichia coli*, S.A.: *Staphylococcus aureus*, S.F.: *Streptococcus faecalis*, K.P.: *Klebsiella pneumoniae*, S.K.: *Salmonella* Kentucky, P.A.: *Pseudomonas aeruginosa.* a-c bars, within a bacterium, lacking a common letter, differ (P<0.05).

The antibacterial activity was assessed using the inhibition zone, which was measured in millimeters and included the diameter of the paper disk.

The extracts of leaves, flowers, and aerial parts of *B. cinerea* exhibited potent activity against the Gram-positive bacteria *Staphylococcus aureus* (S.A.), with inhibition zones greater than 20 mm, and exceed that of the Gentamicin (14± 0.93 mm). These extracts also showed a high antibacterial effect, compared to that of the positive control against *Streptococcus faecalis* (S.F.). Among the known resistant Gram (-) bacteria, *Klebsiella pneumoniae* (K.P.) and *Escherichia coli* (E.C.) seem to be moderately sensitive to the extracts tested except stems extract. A medium to weak inhibition effect was observed against *Salmonella Kentucky* (S.K.) and *Pseudomonas aeruginosa* (P.A.).

No significant difference was recorded regarding the inhibition diameters between leaves extract and antibiotic against S.F., K.P., E.C., and S.K.

3.4.2. Antibacterial test with the microdilution method.

To compare the sensitivity of the bacterial strains to the studied extracts. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) were determined, and the results were illustrated in Table 3.

Table 3. MIC and MBC of extracts from different parts of *B. cinerea*.

Bacteria	Lv		Fl		AP		St	
	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
S.A	0.78	0.78	1.56	1.56	1.56	3.13	3.13	3.13
S.F	0.78	1.56	3.13	3.13	3.13	3.13	12.5	12.5
K.P	3.13	3.13	6.25	6.25	6.25	12.5	25	25
E.C	6.25	6.25	12.5	12.5	12.5	25	25	50
S.K	6.25	6.25	25	50	50	50	*	*
P.A	25	50	*	*	*	*	*	*

*No effect, Fl: Flower extract, Lv: Leave extract, St: Stem extract, AP: Aerial part extract, E.C.: *Escherichia coli*, S.A.: *Staphylococcus aureus*, S.F.: *Streptococcus faecalis*, K.P.: *Klebsiella pneumoniae*, S.K.: *Salmonella* Kentucky, P.A.: *Pseudomonas aeruginosa.*

The aqueous extracts of different parts of *B. cinerea* plant inhibit the growth of Gram-positive bacteria at a concentration ranging from 0.78 to 12.5 mg/ml. While the MIC against Gram-negative bacteria varies between 3.13 and 50 mg/mL, except for the *P. aeruginosa*, which showed stiff resistance to all concentrations of flowers, stems, and aerial parts extracts. Moreover, the studied extracts exhibit a bactericidal effect against the susceptible bacteria with a ratio of MBC/MIC less than 4.

Bensizerara *et al*. [71] described the interference of ethanol, n-butanol, ethyl acetate, and petroleum ether extracts of the aerial part of *B. cinerea*, and elicited antibacterial activities against *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli*.

Antibacterial activities depended on extracts, their concentrations, and bacteria. In previous studies, similar results were obtained from different plant extracts.

Efenberger-Szmechtyk *et al*. [72], Okla *et al*. [73], Deans and Svoboda [74], Özcan and Boyraz [75], Sagdıç *et al*. [76], and Chlif *et al*. [77] found that the antibacterial activity may depend on the type, composition, and concentration of the extracts, as well as on the type of the targeted bacteria and the studied plant organ.

The present work results indicate that the different extracts varied in response to Gram-positive (S.A. and S.F.) and Gram-negative bacteria (E.C., S.A., S.F., K.P., S.K., and P.A.). This is in concord with previous studies, showing that most Gram-negative bacteria are more resistant to antibacterial agents [78,79].

Gram-positive bacteria are considered more susceptible to different antibacterial compounds due to the structure of their cell walls [80,81].

The phytochemical screening results showed that the extracts of different parts of *B cinerea* contained some phytochemical compounds that had a good effect on the tested bacterial strains. Leaves extract had the best antibacterial properties. This finding conforms with the work of Sikandar *et al*. [82], who also reported the effect of these phytochemicals as a good antibacterial agent on different tested microorganisms.

In this regard, Onwuliri and Wonang [83] reported that phytochemical compounds such as saponins allowed the entry of toxic material or leakage of vital constituents from the cell, while tannin coagulates the cell wall proteins.

Flavonoids inhibit the enzyme activity by forming complexes with bacterial cell walls, extracellular and soluble proteins. More lipophilic flavonoids disrupt low concentrations of cell wall integrity [84] or microbial membranes [85]. Alkaloids are the most efficient therapeutically influential plant substance. Pure natural or synthetic alkaloid derivatives are used as a basic medical agent because of their antibacterial analgesic and antispasmodic properties [86]. Alkaloids in *B. cinerea* show that this plant can be an effective anti-malaria agent since alkaloid consists of quinine, which is anti-malaria [87].

Marchese and Shito [88] and Poole [89] reported the sensitivity of the bacterial strains to both the plant extracts and synthetic antibiotics and observed that the plant extracts compete with the drugs and can be used as an alternative to the antibiotics since the inhibition zones were very comparable, and the extracts have fewer side effects than antibiotics [90].

4. Conclusions

This work is the first report on the phytochemical screening, antioxidant and antibacterial activities of leaves, flowers, stems, and aerial parts of *B. cinerea plant* growing in southeastern Morocco.

The result of this study shows that among the plant organs studied, leaves extract exhibits the highest polyphenols, flavonoids, and tannins contents and the most potent antioxidant and antibacterial activities, whereas stems extract presented the lowest ones.

Interestingly, the different extracts' antioxidant activity was positively correlated with their phenolic contents, and the antibacterial activity positively correlated with their flavonoid and tannins contents.

With promising antioxidant and antibacterial properties, *B. cinerea*, and particularly its leaves, can be an inexpensive source of natural substance for use in pathogenic systems to prevent the growth of bacteria, extend the shelf life of the processed foods, and in pharmaceutical industries.

However, further investigations are necessary to evaluate the toxicity level of these Moroccan plant extracts and develop an optimized dose to maintain the product's safety and shelf-life.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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