

Original Research Article

PHYTOCHEMICALS PROFILING, TOTAL PHENOLICS AND TOTAL ANTIOXIDANT CONTENT OF EXTRACTS OF DIFFERENT PARTS OF *GRANGEA MADERASPATANA* FROM BURKINA FASO

Modify the title as described below ;

Physiochemical Analysis, Total Phenolics Quantity And Antioxidants Potential of *Grangea Maderaspatana* From Burkina Faso

Abstract

This work is a contribution to a better use of *Grangea maderaspatana* belong to family Asteracea as a medicinal plant for its phytochemicals. Extracts of different parts of *Grangea maderaspatana* were thus screened for their phytochemicals, including phenolics, tannins and flavonoids contents and total antioxidant contents.

The extracts were obtained from the roots, flowers, leafy branches and the whole plant using hexane, dichloromethane, ethyl acetate and methanol. The phytochemical screening was carried out by means of thin layer chromatography, colorimetric and precipitation reactions. Total phenolics contents, total flavonoids et total antioxidants contents were evaluated by spectrophotometric methods.

The phytochemical screening revealed that *G. maderaspatana* different parts contained phenolics, tannins, flavonoids, alkaloids, sterols and terpenes at different content. Total phenolics contents ranged from 0.051 to 93.455 mg GAE/g of extract in dichloromethane extract of leafy branches and in the ethyl acetate extract of flowers, respectively. Those of total flavonoids ranged from 82.984 in the roots methanolic extract to 753.337 mg QE/g in the ethyl acetate extract of flowers. Total antioxidant contents, assessed by *DPPH*, *FRAP* and *ABTS*⁺ methods are highest in ethyl acetate extracts compared to others extraction solvents.

G. maderaspatana contains a variety of secondary metabolites whose levels vary according to the plant part used.

Keywords: *Grangea maderaspatana*, different organs, phytochemicals contents, total antioxidants.

1. INTRODUCTION

Plants are known for their ability to produce bioactive substances. These substances are generally secondary metabolites, the most well-known of which are tannins, flavonoids, alkaloids, sterols and terpenes. These different groups of compounds are increasingly sought by the food, pharmaceutical and cosmetics industries [1] because of their multiple biological properties, particularly their antioxidant properties. Medicinal plants have become a worldwide topic drawing an impact on world health. Herbal medicine has played a crucial role in the maintenance of the healthcare system of the wide population throughout the world. The most dominant family reported in terms of medicinal plants was Asteraceae [2-4]. The interest in these natural molecules with antioxidant properties is mainly due to the fact that synthetic antioxidants are being questioned because of their toxicity risk. In higher plants, these secondary metabolites can be found in different organs (leaves, flowers, roots, stems) at varying levels.

Grangea maderaspatana is an aromatic plant traditionally used to treat several pathologies: eye and ear pain, headaches [5], hepatitis, muscle and joint pain [6]. Its roots are known to be an aperitif, intestinal astringent, diuretic, anthelmintic, emmenagogue and galactagogue [6,7].

Previous scientific work showed that *G. maderaspatana* has numerous pharmacological properties including analgesic, diuretic [8,9], hepatoprotective [10], anti-nociceptive, anti-inflammatory, anti-rheumatic [5,11], anti-anxiety, and breast cancer [12]. The essential oil of the plant was found to be a good source of antioxidants with excellent antimicrobial properties [13,14].

Despite the multiple properties attributed to this plant, few works, to our knowledge were reported on the phytochemicals and the antioxidant properties of extracts of the different organs of this plant acclimatised in Burkina Faso. The present work is therefore a contribution to a better knowledge and better use of this plant acclimatised in Burkina Faso ; it aims to carry out a phytochemical study and an evaluation of the antioxidant activities of the extracts of the different organs of *G. maderaspatana* with a view to its valorisation as a natural source of antioxidants.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material included different parts of *G. maderaspatana* (flowers, roots, leafy branches) and the whole plant. The plant is collected in Ouagadougou, around the dam N°2 of this said city. The flowers, roots and leafy branches are separated, dried in a dark place and then ground into powder.

The Plant was identify by Plant Taxonomist----- ; and voucher specimen was deposited at the herbarium-----

2.2. Methods

2.2.1. Preparation of extracts

The extracts were obtained by successive exhaustion using solvents of increasing polarity: hexane, dichloromethane, ethyl acetate and methanol. Indeed, 100 g of each organ were macerated with sufficient quantities of solvents to avoid saturation effect. All extractions were repeated in triplicate. The filtrates were combined and concentrated in the rotary evaporator to the minimum volume under reduced pressure at a temperature of 35°C. The concentrated extracts were then transferred to petri dishes and dried in an oven at 37°C. Thus, sixteen (16) crude extracts were obtained, four for each part of the plant.

2.2.2. Phytochemical screening (Physiochemical not phytochemical)

The different extracts obtained were redissolved in their extraction solvents and thus subjected to phytochemical screening using the methods described by Wagner et al. [15]. The screened classes of compounds were flavonoids, tannins, alkaloids, saponosides, sterols and terpenes.

2.2.3. Total phenolics content

Total phenolics contents (TPC) of the extracts were determined by the method described by Singleton and Rossi [16] using Folin-Ciocalteu reagent.

In this method, 60 µL of Folin-Ciocalteu reagent (FCR) were added to 60 µL of suitably diluted extract. The mixture was left at room temperature for eight minutes to allow complete reaction of the RFC on oxidisable substances or phenolates. Then 120 µL of a 7.5% solution of Na_2CO_3 is added to neutralise the residual reagent. Absorbances were recorded at 760 nm with a microplate reader (spectrophotometer MP96, SAFAS) after incubation at 37 °C for 30 minutes. The results, determined from the equation of a calibration curve ($y = 21.298x + 0.354$; $R^2 = 0.9956$) established from the gallic acid taken as reference, were expressed in mg of Gallic Acid Equivalent per gram of extract (mg GAE/ g).

2.2.4. Total flavonoids contents

Total flavonoids contents of the extracts were assessed according to the colorimetric method described by Zhishen et al. [17]. 50 µL of the suitably diluted extracts were mixed with 150 µL of bi-

distilled water, followed by 15 μL of 5% (w/v) NaNO_2 . 5 min later, 15 μL of a 10 % (w/v) aluminium trichloride solution is added. The mixture was allowed to stand at room temperature for 6 min. Then, 50 μL of a 1 N sodium hydroxide solution were added and the absorbance of the pinkish mixture, was measured at 510 nm using a microplate reader (spectrophotometer MP96, SAFAS). A calibration curve was established using quercetin as a reference according to the same procedure as the sample. Flavonoid contents of the extracts, expressed in mg of Quercetin Equivalent per gram of extract, were calculated by relating the absorbances values on the calibration curve ($y = 0.7932x + 0.0025$; $R^2 = 0.9958$).

2.2.5. Total antioxidant content

Total antioxidant contents were assessed by three methods: the DPPH \cdot test, the ABTS $^{+\cdot}$ test and the ferric ion reducing power (FRAP).

2.2.5.1. DPPH \cdot test

Antioxidant content were determined by the method described by Lamien-Meda et al. [18]. Stock methanolic solution of DPPH \cdot radical $10^{-4} \text{ mol.L}^{-1}$ was first prepared. Then 50 μL of suitably diluted extracts were mixed to 200 μL of the methanolic solution of DPPH \cdot . The resulted mixture was shaken and then stored at room temperature and protected from light. The absorbances were read at 517 nm using a microplate spectrophotometer (MP96 spectrophotometer, SAFAS) 10 minutes after incubation. The absorbances read were related to the calibration curve ($y = -18.685x + 0.6782$; $R^2 = 0.9932$) previously established using trolox as standard antioxidant. The results were expressed in milligrams of trolox equivalent per gram of extract (mg TE/g). All measurements were performed three times.

2.2.5.2. The FRAP method

In this method as described by Pulido et al. [19], a ferric salt, Fe (III) (TPTZ) $_2\text{Cl}_3$ (TPTZ = 2,4,6-tripyridyl-s-triazine) was used as the oxidant. 20 μL of suitably diluted extracts were mixed with 30 μL of distilled water and then 200 μL of FRAP reagent were added. The absorbance of the intense blue coloration of the resulted mixture was measured at 595 nm using the microplate reader (spectrophotometer MP96, SAFAS) 10 minutes after incubation at room temperature. Previously, a calibration curve ($y = 20.062x + 0.2805$; $R^2 = 0.9965$) was established using trolox as reference antioxidant. The absorbance values read were related to the calibration curve equation and the results were expressed as milligram of trolox equivalent per gram of extract (mg TE/g). All measurements were repeated three times.

2.2.5.3. ABTS $^{+\cdot}$ test

The method described by Miller and Rice-Evans [20] was used. Trolox was taken as a reference antioxidant. A calibration curve was first established. 50 μL of each suitably diluted extract were added to 200 μL of ABTS $^{+\cdot}$ solution. The mixture was stirred and kept at room temperature, protected from light. The absorbance decay was measured at 734 nm using a microplate spectrophotometer (spectrophotometer MP96, SAFAS). The antioxidant content of the extracts as determined by relating the absorbances values to the equation of the calibration curve ($y = -18.196x + 0.3455$; $R^2 = 0.9967$) previously established from the trolox taken as standard. Thus, the results were expressed in milligrams (mg) of trolox equivalent per gram of extract (mg TE/g). All measurements were performed in triplicate.

2.3. Statistical analysis

Quantitative data analysis was performed using IBM SPSS Statistics, version 25.0.0. The extract assay experiments were performed in triplicate. Results were expressed as mean \pm standard deviation. P Values < 0.005 are considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

The results of the phytochemical screening using test tubes and chromatographic methods are reported in Table 1.

All the prepared extracts contained flavonoids, sterols and terpenes. In contrast none of plant part contained saponosides. Tannins were present in methanol, ethyl acetate and dichloromethane extracts of all parts, except the roots dichloromethane extracts. As for alkaloids, their presence is variable from an extract to another and from an organ to another. This work is consistent with that of Tanvi et al. [21,22] in India and Dabiré et al. [14] in Burkina Faso, who reported respectively the presence of tannins, flavonoids, sterols and terpenes in extracts from leafy branches and the whole plant of *G. maderaspatana*. In contrast to Tanvi, the work of Dabiré et al. [1141] corroborated our own work and indicated the presence of alkaloids in the leafy branches of the plant collected in Burkina Faso. These results suggest that all the extracts obtained had significant antioxidant properties, especially those of ethyl acetate, given its richness in phenolic compounds. The presence of these different secondary metabolites could justify the therapeutic properties attributed to the plant. Similarly, the particular presence of alkaloids in these extracts would confer a particular biological activity to the plant.

Previous studies have been conducted on the leafy branches and the whole plant. [21, 22]. The present study takes into account all parts of *G. maderaspatana* obtained separately. Thus, to our knowledge it would be reported for the first time.

Table 1. Chemical groups found in extracts of the different organs of *G. maderaspatana*

Plant organ	Extract	Chemical groups				
		Tannins	Flavonoids	Alkaloids	Saponosides	Sterols-terpenes
Whole plant	Hexane	-	+	+	-	+
	DCM	+	+	-	-	+
	EtOAc	+	+	+	-	+
	MeOH	+	+	-	-	+
Leafy branches	Hexane	-	+	+	-	+
	DCM	+	+	+	-	+
	EtOAc	+	+	-	-	+
	MeOH	+	+	+	-	+
Flowers	Hexane	-	+	+	-	+
	DCM	+	+	+	-	+
	EtOAc	+	+	+	-	+
	MeOH	+	+	-	-	+
Roots	Hexane	-	+	-	-	+
	DCM	-	+	+	-	+
	EtOAc	+	+	-	-	+
	MeOH	+	+	-	-	+

- : absence ; + : presence

3.2. Total phenolics content (TPC)

The values of TPC are reported in Table 2. Statistical analysis shows that the total phenolic contents varied significantly ($p < 0.05$).

The analysis of the results in Table 2 showed that phenolics are more or less important in the different extracts, depending on the extraction solvent and the organ. Ethyl acetate was found to be the suitable solvent to extract phenolics in *G. maderaspatana* different organs.

TPC varied from 0.051 mg GAE/g in the dichloromethane extract of the leafy branches to 93.455 mg GAE/g in the ethyl acetate extracts of the flowers. Ethyl acetate extract contained the highest contents in both the organs (separately) and the whole plant, followed by methanolic extracts. Among the ethyl acetate extracts, the flowers have the highest content (93.455 mg GAE/g) and the leafy branches the lowest (57.921 mg GAE/g). In the methanol extracts, the whole plant has the highest content and the leafy branches the lowest.

This study suggest that it is better to use only flowers of *G. maderaspatana* instead of the whole plante to get more phenolics.

Table 2. Total phenolics content (TPC) of *G.maderaspatana* extracts

Extract	Total Phenolics content (mg GAE) /g extract			
	Whole plant	Leafy branches	Flowers	Roots
Hexane	12.113±0.019 ^{cde}	7.506±0.287 ^{bc}	4.536±0.066 ^{ab}	4.442±0.075 ^{ab}
DCM	0.063±0.001 ^a	0.051±0.001 ^a	8.996±0.248 ^{bcd}	7.600±0.646 ^{bcd}
EtOAc	59.198±1.479 ^g	57.921±0.075 ^g	93.455±4.958 ^h	61.602±3.261 ^g
MeOH	26.974±2.155 ^f	12.260±0.047 ^{cde}	12.798±0.254 ^{de}	15.860±0.707 ^e

The means in each column followed by a different letter are significantly different ($p < 0.05$)

3.3. Total flavonoid content (TFC)

The total flavonoid contents are gathered in Table 3. These contents are statistically significant ($p < 0.05$) and variable according to the organ and the extraction solvent. They vary from 82.984 in the roots methanolic extract to 753.337 mg QE/g in the ethyl acetate extract of the leafy branches. Ethyl acetate extracts were the richest in flavonoids. Indeed, the flowers extract has the highest content with 753.337 mg QE/g, followed by the leafy branches with a content of 681.795 mg EQ/g. The content of roots were 641.45 mg QE/g and the whole plant has the lower content with 474.701 mg QE/g.

Table 3. Total flavonoid content of *G. maderaspatana* extracts

Extract	Total flavonoid content (mg QE / g of extract)			
	Whole plant	Leafy branches	Flowers	Roots
Hexane	196.672±10.086 ^{ef}	144.982±12.607 ^{cd}	138.678±5.043 ^{bc}	109.472±5.685 ^{ab}
DCM	165.406±6.051 ^{cde}	207.766±20.171 ^f	191.629±4.034 ^{ef}	156.665±14.168 ^{cd}
EtOAc	474.701±18.633 ^g	681.795±8.068 ⁱ	753.337±12.111 ^j	641.45±8.068 ^h
MeOH	176.10±3.557 ^{def}	147.571±11.521 ^{cd}	155.705±8.703 ^{cd}	82.984±1.824 ^a

The means in each column followed by a different letter are significantly different ($p < 0.05$)

In view of the results obtained, it should be noted that total phenolics and flavonoids contents vary according to the solvent used and the plant organ. Similar observations have been reported in other works. Indeed, while working on solvents influence on the total phenolics contents of *Rhanterium adpressium* extracts, Chahrazed et al. [23] found that the amount of total phenolics is variable from one solvent system to another and from one extract to another.

In our work, the ethyl acetate extracts recorded the highest contents of total phenolics and flavonoids, methanolic extracts follow after ethyl acetate extracts for total phenolics contents. These results are in agreement with the literature. Indeed, in a liquid-liquid extraction with water-immiscible solvents, ethyl acetate entrains the majority of the heterosides [24]. Similar results have been reported by some authors who have mentioned the fact that the extraction system influences the total phenolics content. Thus, by measuring total phenolics in onion (*Allium cepa* L.) varieties, Rasmané et al. [25] showed that the ethyl acetate extracts contained the highest total phenolics contents compared to ethanol/water (70/30) and acetone/water (80/20). The work of Chahrazed et al. [24] on the determination of total phenolics and flavonoids in *Rhanterium adpressium* extracts showed that the ethyl acetate extracts contained the highest of total phenolics and total flavonoids contents compared to the chloroformic, hexanic and butanolic extracts.

We note that total phenolics and flavonoids contents are dependent on the extraction solvent and unevenly distributed in the organs. This unequal distribution has been reported by authors [26]. The aerial parts have high phenolics and flavonoids contents. This can be explained by the fact that the leafy branches and flowers are the most exposed to sunlight. Indeed, flavonoids protect plant tissues from the harmful effects of solar radiation [26]. This could be related to the harsh climatic conditions of the places where they grow (high temperature, high sun exposure, drought and salinity) which

stimulate the biosynthesis of secondary metabolites such as polyphenols [27]. Few bibliographic data concerning total phenolics and flavonoids contents on *G. maderaspatana* extracts are available. Nevertheless, some more or less recent works have been reported. Thus, Bationo et al. [28] indicates about 194.554 mg GAE/g in a methanolic extract of leafy branches ; this is largely superior to ours, which is about 12.260 mg GAE/g of extract. Dabiré et al [14] also determined total phenolics content in the leafy branches of *G. maderaspatana* of extracts obtained from hydroalcoholic solutions (ethanol 30%, 60%, 96%) and by decoction. They obtained the contents of 0.919; 0.839; 0.271; 2.013 mg GAE/g plant material respectively. Also, Kaushal et al. [29] recorded a content of 121.45 mg GAE/g plant material in the methanolic extract of the whole plant. However, it is difficult to make a rigorous comparison because of the different extraction methods.

These different results could also show that several factors influence the total phenolics content. Studies have shown that extrinsic factors such as climatic and geographical factors, genetic factors, also the degree of ripening and storage time have a considerable influence on the total phenolics content [23].

These results corroborate the phytochemical screening and show that the different extracts of *G. maderaspatana* organs contain significant amounts of phenolics and flavonoids, especially those with ethyl acetate, particularly in the flowers and roots. This plant could be a potential source of phenolic compounds and its ethyl acetate extracts should have better antioxidant activities.

3.4. Total Antioxidant Content (TAC)

The results of the total antioxidant content are given in Table 4. Statistical analysis showed that total antioxidant content varied significantly ($p < 0.05$) according to the plant organs and the extraction solvent.

These results showed that TAC of whole plant varied from 0.320 with the hexanic extract to 10.068 mg TE/g with the ethyl acetate extract by the ABTS method. By the DPPH method, they range from 3.615 to 24.393 mg TE/g for the hexane and ethyl acetate extracts respectively.

For the leafy branches, by the DPPH method, the contents are between 1.254 with the hexane extract and 13.209 mg TE/g with the ethyl acetate extract. Contents between 3.987 and 88.844 mg TE/g are recorded for the DCM and ethyl acetate extracts respectively by the FRAP method. By the ABTS method, they are between 0.458 for the DCM extract and 8.309 for the ethyl acetate extract.

For the flowers of *G. maderaspatana*, it appears that by the DPPH method, the contents vary from 2.669 with the DCM extract to 10.698 mg TE/g with the ethyl acetate extract. By the FRAP method, they range from 5.184 to 72.468 mg TE/g for the hexane and ethyl acetate extracts respectively. The ABTS method reveals that these contents are between 2.022 for the DCM extract and 12.618 mg TE/g for the ethyl acetate extract.

The TAC in the roots is also a function of the extracts and the methods used. In fact, by the DPPH method, these contents vary from 3.341 for the hexane extract to 12.325 mg TE/g for the ethyl acetate extract. By the FRAP method, they range from 6.513 for the DCM extract to 92.566 mg TE/g for the ethyl acetate extract. By the ABTS method, they vary between 1.165 and 12.003 mg TE/g for the DCM and ethyl acetate extracts respectively.

By all three methods and for the organs, the contents of the hexane or DCM extracts are the lowest while those in ethyl acetate are the highest. These results are in agreement with those above (phytochemical screening and of total phenolics and flavonoids contents in the ethyl acetate extracts). By the DPPH and FRAP methods, the best contents are found with the whole plant while by the ABTS method, they are high with the flowers. Comparing the contents of the ethyl acetate extracts of the different organs, it can be seen that the leafy branches have the highest content by the DPPH method while by the FRAP method, the highest content is observed with the roots. By the ABTS method, the flowers have the highest content.

As the results indicate, all the extracts show variable antioxidant activity. However, all are capable of reducing free radicals and iron ions. This work corroborates the work of Kaushal et al. [29] and Varsha et al. [30] in India who showed that methanolic extracts of the plant have significant antioxidant activity. However, it is difficult to make a rigorous comparison with our work because the extraction methods of these authors are different from ours.

From these results, we note that the ethyl acetate extracts are the most antioxidant-rich and their contents vary from one organ to another.

Table 4. Total antioxidant contents (TAC)

Plant	Extracts	TAC (mg TE /g extract)
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material		DPPH	FRAP	ABTS
Whole plant	Hexane	3.615±0.285 ^b	3.376±0.184 ^a	0.320±0.032 ^{ab}
	DCM	5.811±0.215 ^c	8.055±0.422 ^a	0.755±0.025 ^{bc}
	EtOAc	24.393±0.178 ⁱ	111.973±5.742 ^d	10.068±0.264 ^g
	MeOH	19.149±0.264 ^h	21.708±2.074 ^b	3.324±0.051 ^e
Leafy branches	Hexane	1.254±0.067 ^a	8.374±0.319 ^a	-
	DCM	5.338±0.528 ^c	3.987±0.079 ^a	0.458±0.033 ^{ab}
	EtOAc	13.209±0.332 ^g	88.844±5.104 ^d	8.309±0.152 ^f
	MeOH	11.355±0.894 ^{ef}	25.043±1.047 ^b	2.529±0.077 ^d
Flowers	Hexane	5.25±0.267 ^c	5.184±0.159 ^a	-
	DCM	2.669±0.082 ^b	5.264±0.079 ^a	2.022±0.176 ^d
	EtOAc	10.698±0.108 ^e	72.468±1.328 ^c	12.618±0.577 ⁱ
	MeOH	8.289±0.473 ^d	24.853±1.334 ^b	2,327±0,195 ^d
Roots	Hexane	3.341±0.157 ^b	20.416±1.276 ^b	-
	DCM	4.867±0.041 ^c	6.513±0.332 ^a	1.165±0.088 ^c
	EtOAc	12.325±0.09 ^g	92.566±9.165 ^d	12.003±0.233 ^h
	MeOH	11.286±0.174 ^{ef}	25.718±2.240 ^b	2.282±0.046 ^d

-.not found. Different letters correspond to significant difference ($p < 0.05$)

The results showed that TAC varied according to the polarities of the extraction solvents, organs and methods used. The highest contents, for each of the three methods (*DPPH*, *FRAP* et *ABTS*⁺), were noticed in the ethyl acetate extract, followed by the methanol extract. Total phenolics contents were also the highest in these two extracts. It is well known that phenolic compounds have a strong antioxidant capacity due to the position of their double bonds and the quantity of hydroxyl groups. Thus, there is a strong correlation between the content of phenolic compounds and antioxidants. This work is in agreement with Kaushal et al. [29] who attributed the antioxidant capacity to the phenolic and flavonoid compounds present in the extract. As recommended by Qahtani et al [30] the plant can not be recommend to use directly for medicinal purposes before the proper investigation of heavy metals too. Because the amount of heavy metal vary from place to place depend upon the geographical condition.

4. CONCLUSION

Grangea maderaspatana different parts contained several compounds, notably phenolics, which were found to have significant levels, alkaloids, sterols and terpenes. The results show that total antioxidant levels are closely related to phenolic levels. The ethyl acetate extracts of the plant organs and the whole plant are the most active and show considerably higher total phenolics and flavonoids contents. Also, the phytochemical composition and contents of the extracts vary from one extract to another and from one organ to another. The organs of *G. maderaspatana* could be a potential source of natural antioxidant. Further studies are needed to isolate and identify the molecules responsible for this antioxidant activity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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