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Original Research Article

Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Euphorbia resinifiera* O. Berg

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ABSTRACT

Euphorbia resinifiera is a well-known medicinal plant used by the Moroccans to treat several diseases. This study evaluated the influence of extraction solvents polarities (methanol, aqueous/methanol (3:7), and distilled water) on the polyphenols and flavonoids contents of *Euphorbia resinifiera* extracts obtained by three extraction methods: infusion, maceration and sonication, as well as their antioxidant activity. The results revealed that the distilled water extract obtained by infusion presented the highest polyphenols and flavonoids contents: 8.89 ±0.01 mgGEA/g dry weight, and 4.98±0.01 mgRE/g dry weight, respectively. The antioxidant activity results revealed that the aqueous-methanol extracts showed the highest antioxidant activity. These findings could support the medical uses of *Euphorbia resinifiera*.

Keywords:*Euphorbia resinifiera*, Extraction method, Polyphenols, Flavonoids, Antioxidant Activity.

Introduction

Since ancient times, humans have utilised plants for different needs, particularly for health care and the food industry. Today, nature remains a reliable source of medicinal agents.[1] Approximately 40% of currently available drugs are directly or indirectly derived from natural products, mainly of plant origin.[2] According to the world health organization, around 65-80% of the world's population in developing nations depend on traditional herbal medicines for their primary health care due to poverty and difficulty in accessing modern medicine.[3] In Africa, people still extensively use medicinal plants to cure diseases. Using herbal remedies and other materials is an integral part of African culture.[2]Recently, there has been increased interest in plant chemistry because plants represent an essential source of a wide variety of bioactive molecules used in the food industry, cosmetology, and pharmacy. Some bioactive compounds of plant origin include coumarins, alkaloids, phenolic acids, tannins, terpenes, and flavonoids.[4-5]Also, most medicinal and aromatic plants contain compounds with antioxidant properties.[6] Much research has been done on developing natural antioxidant formulations for use in foods, cosmetics, and other applications.[7-8]Among compounds with antioxidant properties, polyphenols constitute an important group with potent free radicals scavenging activities.[9-10]Antioxidant activity screeningis widely used to characterize dietary supplements and bioactive compounds, and this area of research is trending in the scientific community.[11]

Euphorbia resinifiera O. Berg is an endemic medicinal plant of Morocco, more precisely of the middle atlas. It belongs to the Euphorbiaceae family.

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According to Moroccan herbalists, *Euphorbia resinifiera* has been used for treating several diseases, but this claim is yet to be validated scientifically. This study aimed to determine the influence of extraction solvent polarity on the polyphenolic and flavonoids content of the aerial part of *Euphorbia resinifiera* and its antioxidant activity using different assay methods.

Materials and Methods

Plant collection and preparation

Euphorbia resinifiera was collected in February 2020 in Azilal province, Morocco. The plant was identified at the Department of Botany and Plant Ecology, Scientific Institute Rabat, Morocco. A voucher specimen (N RAB113340) was deposited at the Herbarium of the same institute. The plant material was dried at 30°C, grounded and sifted.

Extracts preparation of Euphorbia resinifiera

The dried and powdered plant material was extracted by sonication, maceration and infusion.

Sonication

The grounded powder (5 g) was mixed with 50 mL of each solvent: methanol, distilled water, and methanol (70%). The different samples were ultra sonicated in a water bath for 45 min with a break of 10 minutes accordingly. Subsequently, the samples were centrifuged at 3000rpm for 10 min. The supernatant was collected and stored in glass tubes at 4°C until use.[12]

Maceration

The protocol of Lezoul *et al*.[13]was adopted with some modifications. The powder sample (5 g) was mixed with 50 mL of each extraction solvent (methanol, distilled water, and aqueous-methanol (70%)). The extracts were filtered with Whatman No. 1 filter paper, and the filtrates were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and stored in glass tubes at 4°C until use.

Infusion

Infusion extraction was performed according to the protocol described by El hamsas *et al*.[14] The powder (2.5 g) material was placed in a flask containing 25 mL of boiling distilled water and left to cool at room

temperature. The filtrates were centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and stored in glass tubes at 4°C until further analysis.

The total polyphenols content
With some modifications, the amount of total polyphenols contents in the extracts was determined by the Folin-Ciocalteu assay.[15]50 µL of the supernatants was mixed with 450 µL of Folin-Ciocalteu reagent (1:10), and after 5 minutes, 450 µL of sodium carbonate (75 g/L) was added to the mixture. After 5 hours of incubation in the dark at 25°C, the absorbance was measured at 750 nm. In the same conditions, gallic acid was used as a standard to establish the calibration curve, and the total phenolics contents were expressed as mgGEA/g dry weight.

The total flavonoids content
The total flavonoids contents were determined using the method described previously by Yildirim *et al.*[16]Briefly, 50 µL of each extract was added to 50 µL of methanolic aluminium chloride(10% w/v), and the absorbances were measured at 420 nm after 1 hour of incubation at room temperature in the dark. Rutin was used as a standard to execute the calibration curve. The flavonoid contentswereexpressed as mgRE/g dry weight.

Antioxidant activity
Ferric reducing antioxidant power capacity
The antioxidant activity was evaluated using a Ferric reducing power assay. The assay was performed as described by Togola *et al*[17] with some modifications. Briefly, 250 µL of phosphate buffer (0.2M,pH=6.6) and 250 µL of 1% aqueous potassium hexacyanoferrate solution were added to 50 µL of each sample. After 20 minutes of incubation at 50°C in a water bath, 250 µL of trichloroaceticacid(10%) was added. The mixture was centrifuged at 3000rpm for 10 minutes. 250 µL of the supernatant was collected and mixed with 250 µLdistilled water and 50 µL of aqueous Ferric chloride (0.1% w/v). After incubation for 10 minutes, the absorbances were measured at 700nm using a UV-vis spectrophotometer. Ascorbic acid was used as standard. The antioxidant activity of the extracts was expressed as ferric-reducing power using the following formula:

$PR= [(A_a-A_b)/A_a*100$
Were A_a: Absorbance of the extract, A_b: Absorbance of the blank

Radical DPPH scavenging activity
The free radical capacity of the extract of the extracts was determined as previously described[18] Briefly, 50 µL of the extracts were mixed with 1.95 mL of 63.4 µM DPPH stable free radical prepared in methanol. The mixture was vortexed and incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm with a UV-1800PC UV-vis spectrophotometer against a methanol blank. Ascorbic acid was used as the standard antioxidant agent (positive control). The scavenging activity on the DPPH radical was calculated from the formula as shown.

$DPPH\ scavenging\ effect(\%)=[(A_0-A_1/A_0)]*100$

Were A_0 is the absorbance of the negative control reaction mixture,
$A_1$is the absorbance of test samples/standard.
The antioxidant potential of the extracts expressed as IC_{50} values (the concentration of the extract that produced 50 percent inhibition of the DPPH free radical) was generated from the plot of percentage inhibition versus concentration of the extracts in mg/mL.

Total Antioxidant capacity
Total antioxidant capacity was determined by the slightly modified method.[19] 50 µL of each sample was mixed with 50 µL of molybdate solution (0.6 M). The mixture was thenvortexed and incubated in a water bath for 90 minutes at 90°C. After cooling at room temperature for 10 to 15 minutes, the absorbance was measured at 695 nm. Ascorbic acid was used forthe calibration curve. The total antioxidant capacity was expressed as milligrams of ascorbic acid equivalent per gram of dry weight (mgAA/gDW).

Statistical analysis
Experimental values were expressed as mean ±SD from two independent experiments carried out in triplicates and analysed using MS Excel 2007 statistical software.

Results and Discussion

The polyphenols content of the aerial part of *Euphorbia resinifiera* was determined using the calibration curve of gallic acid ($y=8.208x+0.167$, $R^2=0.993$). The polyphenols content determination results showed that aqueous-methanol (70%) obtained by sonication has the highest value (2.73±0.02 mgGAE/gDW), followed by the methanol extract (2.37±0.03 mgGAE/g DW).In contrast, the water extract has the lowest content (1.30±0.03 mgGAE/gDW) (Figure1). Similarly, the polyphenols content was highest in the aqueous-methanol (70%) extract obtained by maceration (7.09±0.04 mgGAE/gDW), followed by the methanol extract(2.04±0.03 mgGAE/g DW), with distilled water having the least value(Figure2). Also, the total polyphenolic content of the extract obtained by infusion extract gave a value of 8.98±0.01mgGAE/g DW. Studies have shown that the solubility of polyphenols depends principally on the number of hydroxyl groups molecular weight, and the length of the basic skeletal carbon chain.[20] The addition of water to the organic solvent increases the solubility of polyphenols by modulating the polarity of the organic solvent.This increase could be due to the weakness of hydrogen bonds in an aqueous solution. It could also be due to the augmentation of basicity and ionisation of polyphenols in aqueous solutions.[21,22]Another study done by shan *et al*.[23] found that aqueous solvent gives the best content of polyphenols and antioxidant activity compared to alcoholic solvent.

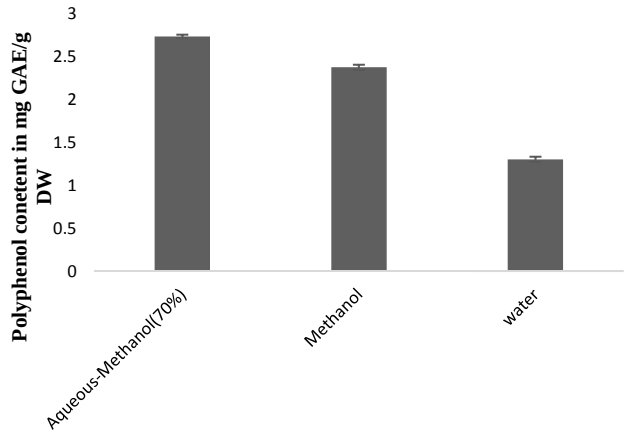

Figure 1: Polyphenol content of *Euphorbia resinifiera* extract using obtained by sonication.

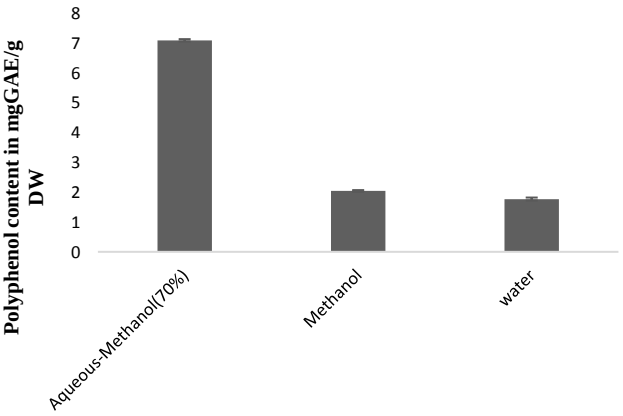

Figure 2: Polyphenol content of *Euphorbia resinifiera* extract obtained by maceration.

Total Flavonoids Content (TFC)

The different extracts' total flavonoid contents were derived from Rutin's calibration curve (y=15.3165X+0.0743; R^2=0.9944). The flavonoids contents of extracts obtained by sonication were 0.51±0.0.03 mg RE/gDW for methanolic extract,which was higher than that of the dist. water extract, but lower than that of the aqueous-methanol (70%) extract (0.56±0.04 mgRE/gDW) (Figure 3).The TFC was, however, higher in the extract obtained by macerating in aqueous-methanol (70%) at 3.51±0.05 mgRE/gDW, followed by methanolic extract (1.02±0.04 mgRE/gDW), and water extracts 0.97±0.05 mgRE/gDW as shown in (Figure4). The total flavonoids content recorded by infusion was 4.98±0.01 mgRE/gDW.

Antioxidant activity screening

Ferric Reducing Antioxidant essay

Evaluation of the reducing power of extracts obtained by sonication showed that the aqueous-methanol (70%) extract possesses the highest antioxidant activity, and the lowest activity was observed in the dist. water extract. Results shown in Figure5 revealed that at the same concentration of 5 mg/mL, the reducing power of the aqueous-methanol (70%) extract, methanolic extract, and Dist. water extract were 95.46±5.7%, 94.30±6.2%, and 92.52±8.8%, respectively. In the extracts obtained by maceration, the highest ferric-reducing antioxidant power was recorded for aqueous-methanol (70%) extract (96.43±1.91%) followed by methanolic extract (94.72±1.83%). The lowest value was recorded by distilled water extract (94.22±1.82%) (Figure6).Similarly, infusion extracts showed the following ferric-reducing antioxidant power values: 92.87±0.90%, 91.41±0.75%, 88.30±1.06%, 84.70±1.87% and 74.34±0.6% at extracts concentrations of 33.33, 16.66, 6.66, 4.76, 3.33 mg/mL, respectively (Figure 7).Ascorbic Acid used as a reference standard showed 95.83±1.2% ferric reducing antioxidant power at a concentration of 33.33mg/mL and 97.54±2.3% at 5 mg/mL, demonstrating strong reducing power potential compared to the crude extracts of *Euphorbia resinifiera*.

Radical Scavenging DPPH activity

The results showed that all tested sonication extracts exhibited potent DPPH free scavenging radical ability(Figure8).The aqueous-methanol (70%) extract has the best DPPH free radical scavengingactivity (87.40±0.37%), followed by methanolic extract(85.88±0.28%), while the distilled water extract has the lowest activity(35.15±0.12%). Ascorbic acid exhibited 97.34±0.17% radical scavenging ability. The IC_{50}values were determined to compare the radical scavenging activity for different sonication extracts of *Euphorbia resinifiera*. The IC_{50} is a measure to establish the antioxidant potential of a test sample. It measures the half-maximal inhibitory concentration of a test sample. It shows how much of a bioactive agent is needed to inhibit a biological process by half. The lower the IC_{50} value, the more potent is the test agent. From this study, the aqueous-methanol extract showed the lowest IC_{50} value of 0.812±0.024 mg/mL compared to the methanol extract of 1.788±0.017 mg/mL. The reference compound Ascorbic acid showed an IC_{50} value equal to 0.083±0.011 mg/mL, which is far more potent than the test extracts(Figure9).All the extracts obtained by maceration showed significant dose-dependent activity. At a concentration of 1.6 mg/mL, the aqueous-methanol (70%) extract has the higher inhibition percentage (93.22±0.1%), followed by methanolic extract(88.13±2.08%). In comparison, the distilled water extract of *Euphorbia resinifiera* recorded the lowest inhibitionpercentage(53.55±3.01%) (Figure10).

The IC_{50}values (Figure11) of the aqueous-methanol(70%), methanolic and water extracts were 0.20±0.014mg/mL,0.55±0.019 mg/mL and 0.85±0.020mg/mL, respectively. In contrast, ascorbic acid showed an IC_{50}value = 0.09±0.015 mg/mL. The infusion extract at the concentrations of 33.33, 16.66, 6.66, 4.76 and 3.33 mg/mL exhibited 66.35±0.29%, 23.47±0.29%, 13.71±2.40%, and 6.43±2.66% percentage inhibition, respectively (Figure12), with an IC_{50}value 25.06±0.23 mg/mL compared to ascorbic acid (2.47±0.14%).

Total Antioxidant capacity

The ascorbic acid calibration curve (y=1.243x+.424, R^2=0.995) was used to generate values for the total antioxidant capacity of the extracts. The aqueous-methanol extract obtained by sonication showed total antioxidant power of 19.13±0.5 mgAA/gDW. This value is higher than the total antioxidant activity of the methanolic extract 8.89±0.6 mgAA/mgDW. The lowest activity was shown by the distilled water extract of *Euphorbia resinifiera* (Figure13).

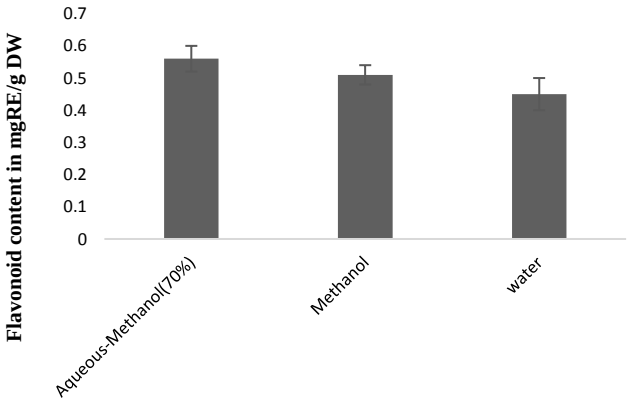

Figure 3: Flavonoids content of *Euphorbia resinifiera* extract obtained by sonication

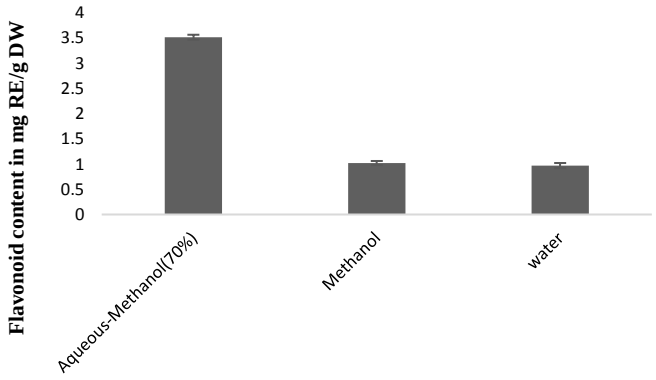

Figure 4: Flavonoids content of *Euphorbia resinifiera* extract obtained by maceration.

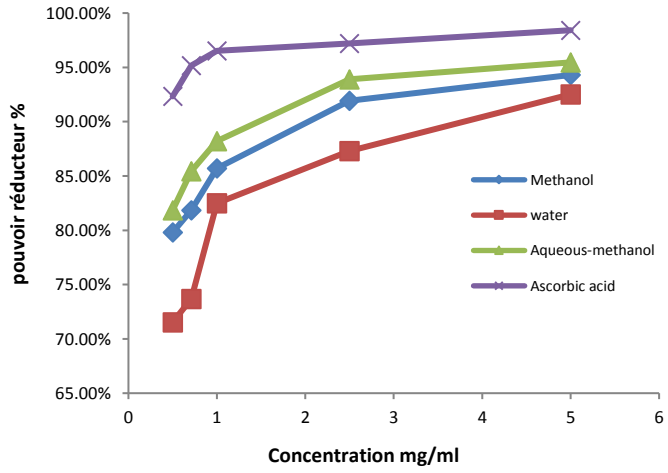

Figure 5: Ferric reducing power potential of *Euphorbia resinifiera* extract obtained by sonication method and ascorbic acid reference

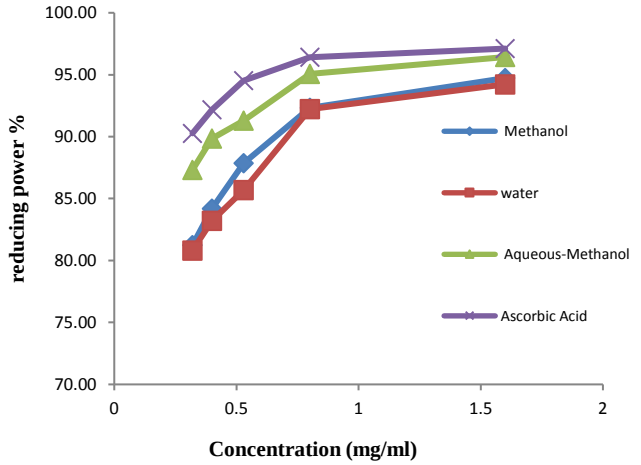

Figure 6: Ferric reducing power potential of *Euphorbia resinifiera* extract obtained by maceration and ascorbic acid reference

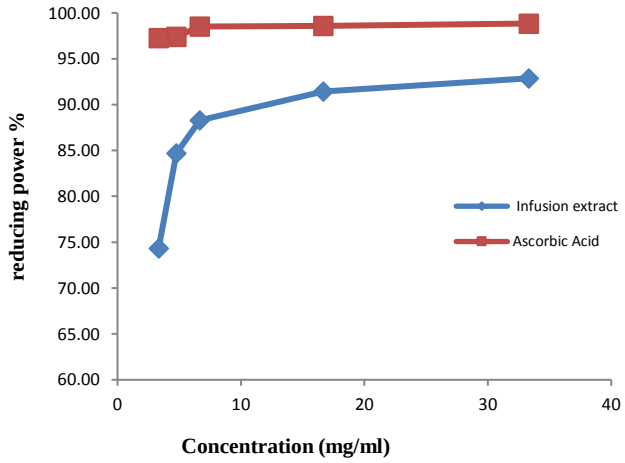

Figure 7: Ferric reducing power potential of *Euphorbia resinifiera* extract obtained by infusion and ascorbic acid reference

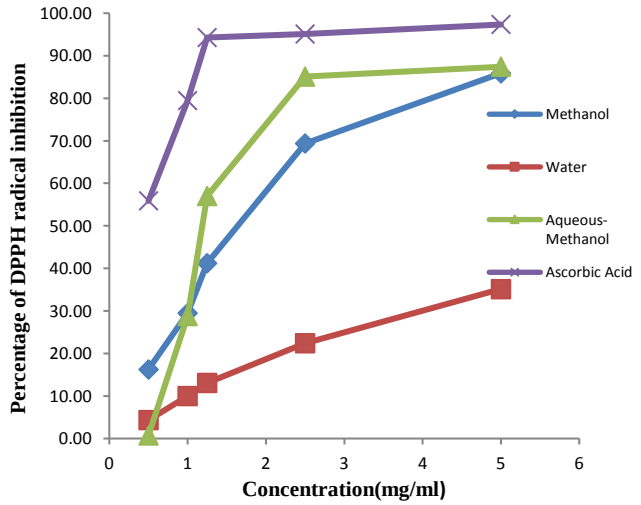

Figure 8: Percentage of DPPH radical inhibition of *Euphorbia resinifiera* extract obtained by sonication and ascorbic acid

Figure 14 showed results fortotal antioxidant capacity for aqueous-methanol (38.21±0.2 mgAA/gDW) and methanolextract(19.34±0.4mgAA/g DW) obtained by maceration. The distilled water extract shows modest antioxidant activity (11.60±0.5 mgAA/gDW). The infusion extract had better total antioxidant activity equal to 133.15±0.05 mgAA/gDW. The infusion extract results are more potent due to the extraction method or the extraction conditions (temperature and extraction time).[24] Notwithstanding the extraction method, the aqueous-methanol (70%) remains the best solvent for extracting polyphenols and flavonoids. Also,the extracts of this solvent show the best free radical scavenging activity, followed by methanol and distilled water.The same observations were reported by Lapornik *et al.*,[20] who showed that the low content of phenolic compounds inaqueous extracts might be due to the absence of oxidases. In contrast, in alcoholic extracts, the enzymes may be active. The different antioxidant activity results could be due to the differences in the vegetal material, solvents utilised for extraction, and the chemical composition of the extracts[25]The method and conditions of extraction (temperature and time) could also affect the antioxidant activity[26] of an extract with a phenolic compound with many hydroxyl groups.[27]

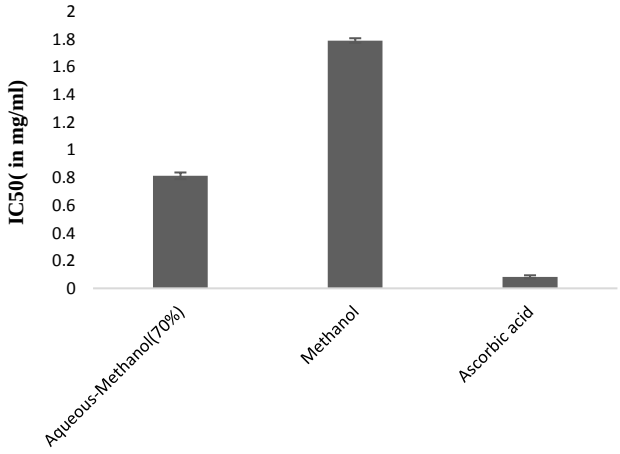

Figure 9: The IC_{50}values of different extracts of *Euphorbia resinifiera* obtained by sonication and the ascorbic acid reference standard.

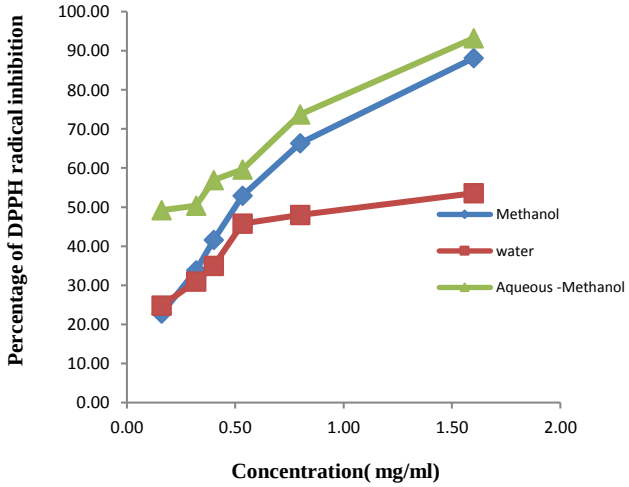

Figure 10: Percentage of DPPH radical inhibition of *Euphorbia resinifiera* extracts obtained by maceration.

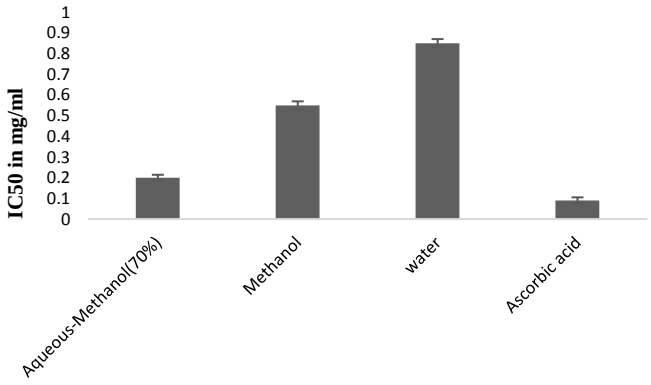

Figure 11: The $IC_{50}$of each extract of *Euphorbia resinifiera* obtained by maceration and ascorbic acid.

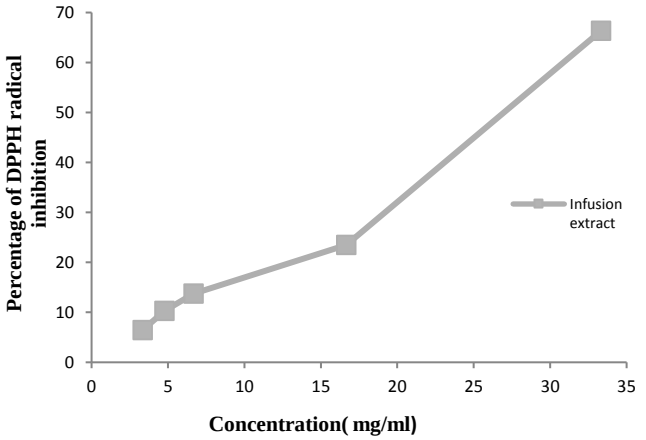

Figure12: Percentage of DPPH radical inhibition of *Euphorbia resinifiera* extract obtained by the infusion method.

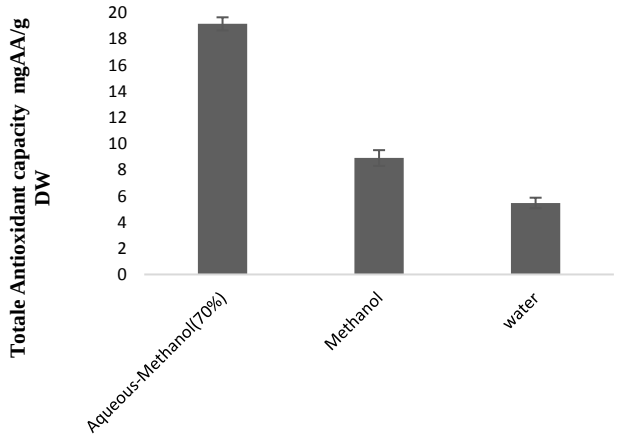

Figure 13: Total Antioxidant Capacity of *Euphorbia resinifiera* extract obtained by sonication method.

Conclusion

The findings of the present study revealed that *Euphorbia resinifiera* has potent antioxidant activity. Furthermore, the results showed that the extraction solvent might significantly affect the contents of polyphenols and flavonoids and the antioxidant activity of *Euphorbia resinifiera* extracts. The aqueous-methanol(70%) showed the highest antioxidant activity, polyphenols and flavonoids contents. However, further studies are required to characterize the compounds responsible for this activity.

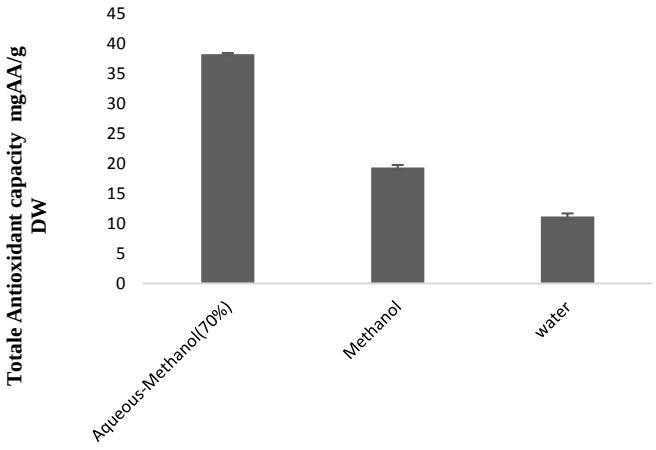

Figure14: Total Antioxidant Capacity of *Euphorbia resinifiera* extract obtained by maceration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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