The Effect of Extraction method and Solvents on yield and Antioxidant Activity of Certain Sudanese Medicinal Plant Extracts

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ABSTRACT

This study came with the objective to compare the effect of extraction method and solvents on yield and antioxidant activity of certain Sudanese medicinal plant extracts, used in traditional medicine for treating various illnesses. The effect of maceration and Soxhlet successive extraction with n-hexane, chloroform and methanol were investigated on the antioxidant activity of five Sudanese medicinal plants. The antioxidant activities were assessed via DPPH (2, 2-Di-(4-tert-octylphenyl)-1-picryl-hydrayl) free radical scavenging activity and Propyl Gallate as standard antioxidants. Maceration was more effective than successive Soxhlet extraction with the same solvents.

Keywords: Sudanese medicinal plants, extraction method and solvents, antioxidant activity.

INTRODUCTION

The Antioxidant is “any substance that delays, prevents or removes oxidative damage to a target molecule” [1]. Antioxidant defense mechanisms are the most effective path to eliminate and diminish the action of free radicals, which cause the oxidative stress [2]. Oxidative stress is a major causative factor in the stimulation of many life threatening diseases, including atherosclerosis, diabetes mellitus, cancer, Parkinson’s disease, immune dysfunction and is involved in premature aging [2-4]. Sudanese plants have been used as medicines for many centuries because they contain active phytochemicals including phenolic compounds. Five Sudanese medicinal plants were selected for the present investigation in the study area White Nile state in Sudan: Euphorbia aegyptiaca, Euphorbia acalyphoides, Francoeuria crispa, Grewia tenax and Cissus quadrangularis.

Euphorbia aegyptiaca and Euphorbia acalyphoides both belonging to the Family Euphorbiaceae, known locally in Sudan as umm lebaina, malbaine. The maceration of the whole plants is used against scorpion bites. The plant of E.aegyptiaca in Sudan used in traditional medicine for treatment of inflammatory conditions like rheumatoid arthritis, conjunctivitis and dermatitis [5]. Euphorbia species contain phytochemical constituents like flavonoids, coumarins, triterpenoids, lignans and alkaloids [6,7].

Francoeuria crispa, syn. Pulicaria crispa, Pulicaria undulata. (Asteraceae) Known locally in Sudan as Rehan, Al-remit or Al-tagar. is an annual herb or sometimes a perennial sub shrub producing small bright yellow flowers. F. crispa is an aromatic herb used in folk medicine for the treatment of inflammation [8] and as insect repellent. Theis poultices of the whole plants are used against alopecia.

The root of Grewia tenax (Tiliaceae), known locally in Sudan as Godhaim, is used to treat jaundice, pulmonary infections and asthma. Leaves are used against trachoma. G. tenaxis is used as medicine to treat various diseases including jaundice and hepatic disorders [9], a decoction prepared from the bark is used as antihelmintic [10]. The fruits, roots and leaves of the plant are used as food while its juice and fruit decoctions have been used in Africa as thirst quenching drinks in hot weather [11]. The fruits are eaten to treat anemia and chest diseases [12].

The Salala is the local name of Cissus quadrangularis, it belongs to the Vitaceae family. The stem and leaves of C. quadrangularis are used in popular medicine the treatment of hemorrhoids, menstruation, scurvy and asthma [13]. Has antioxidant property [14]. Antibacterial and antifungal [15]. Was reported that the plant showed bone fracture healing property [16]. And anti-osteoporotic [17].
Phytoconstituents of *C. quadrangularis* revealed carotenes [15], quercitin [18].

However, to the best of our knowledge, was not investigated before in the Effect of Extraction method and Solvents on yield and Antioxidant Activity of *Euphorbia aegyptiaca*, *Euphorbia acalyphoides*, *Francoeuria crispa*, *Grewia tenax* and *Cissus quadrangularis*. The present study reports our results on the antioxidant activity of extracts prepared by different extraction techniques and different solvents of five Sudanese Medicinal Plants.

### MATERIAL AND METHODS

#### Sample collection

The selected plants were collected from different locations of White Nile state in Sudan figure 1, during February 2016, and were identified in the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. The Voucher specimens were deposited at the herbarium (Table 1). The collected plants were dried for 15 days under the shade, then pulverized by mechanical grinder and stored in well closed glassware containers till usage.

#### Table 1: Summary of Selected Sudanese plants used in traditional medicine

| NO. | Scientific name     | Family           | Local name       | Part used |
|-----|---------------------|------------------|------------------|-----------|
| 1   | *Euphorbia aegyptiaca* Bosss | Euphorbiaceae    | Um lebaina. (malbein) | Whole Plant |
| 2   | *Euphorbia acalyphoides* Hochst.ex. Bosss | Euphorbiaceae    | Um lebaina. (labien) | Whole Plant |
| 3   | *Francoeuria crispa* (forssk.) cass. | Asteraceae.     | Al-tugar.        | Whole Plant |
| 4   | *Grewia tenax* (forssk.) fiori, | Tiliaceae.      | Godhuiam, guddaim | Roots     |
| 5   | *Cissus quadrangularis* L., | Vitaceae.       | Salala           | Whole Plant |

#### Extraction of plant material

Thirty grams of powdered sample of each plant were extracted successively with 400ml n-hexane, chloroform, and methanol. The contents of the conical flask were left at room temperature for 72 h. with frequent shaking.

#### Maceration Method

Thirty grams of powdered sample of each plant using a Soxhlet apparatus were successively extracted with n-hexane, chloroform, and methanol for 48 h. Conditions used to compare Soxhlet and maceration extractions are shown in Table 2, and Physicochemical Properties of Solvents Used in Table 3.

#### Filtration, Evaporation and Yield of extracts

The extracts were filtered using Whatman No. 1 filter paper, the filtered extracts were concentrated by a rotary evaporator, and the residual extracts were dried. The percentage yield was obtained using dry weight, from the equation 1. The extracts were kept and stored in refrigerator at 5 °C until use.

\[
\% \text{ Yield of extract (g/100 g)} = \left(\frac{W_1 \times 100}{W_2}\right)
\]

Where \(W_1\) is the weight of the extract residue after solvent removal and \(W_2\) is the weight of dried plant powder.

#### Antioxidant activity assays

**DPPH radical scavenging assay**

The test was performed according to the method prescribed by Shimada et al. (1992) [19], with some modification. In 96-wells plate, the test samples were allowed to react with DPPH (2, 2-Di (4-tert-octylphenyl)-1-pieryl-hydrazyl) stable free radical for half an hour at 37°C. The concentration of DPPH was kept as (300μM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at \(λ: 517\)nm using multiplate reader spectrophotometer. Percentage radical scavenging activity of samples was determined in comparison with a DMSO treated control group. Propyl gallate was used as a standard antioxidants. All tests and analysis were run in triplicate.

**IC\(_50\) Calculations**

IC\(_50\) the concentration of test material, which possess 50% inhibition of free radicals of all the extracts and their fractions, were determined using monitoring the effect of different concentrations ranging from 0.5-0.0035mg/ml. The IC\(_{50}\) of the extracts and their fractions were calculated by EZ-Fit Enzyme Kinetic Program (Perrella Scientific Inc, U. S.A).

#### Statistical analysis

Data were presented as means ± S.D. Statistical analysis of assays results were performed using the Microsoft Excel program 2013.
RESULTS AND DISCUSSION

Percentage Yields

The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. It will give an idea about the extractability of the plant studied under different conditions. The results were reported in Table 4.

Our results showed that maximum percent yield was obtained when *Euphorbia aegyptiacus* was extracted by maceration technique with methanol (7.123%), followed by chloroform (1.139%) and finally by n-hexane (1.923%). The Soxhlet extraction yielded with methanol (6.137%); followed by Chloroform (0.763%) and finally n-hexane (3.150%).

For the total weight of the *E. acaephyoides* in maceration extraction technique, methanol gave the highest value percentage yield (5.097%); followed by Chloroform (2.164%) and finally n-hexane (1.850%); while the Soxhlet extraction with methanol was 8.456%, followed by Chloroform (1.359%) and finally n-hexane (2.782%).

The total weight of the *Francoeuria crispa* maceration technique with methanol gave 5.610%, followed by chloroform (1.410%) and finally n-hexane (0.711%); while the Soxhlet technique, with methanol gave (8.455%), followed by Chloroform (1.410%) and finally n-hexane (1.458%).

*Grewia tenax* sample when extracted by maceration with methanol gave the highest percentage yield (1.814%), followed by Chloroform (0.315%) and finally n-hexane (0.318%). Soxhlet extraction with, methanol gave (3.623%), followed by Chloroform (0.217%) and finally n-hexane (0.670%).

*Cissus quadrangularis* in maceration extraction technique with methanol gave the highest percentage yield (12.189%), followed by Chloroform 1.154% and finally n-hexane (1.201%); while Soxhlet extraction with methanol yielded 5.464% followed by Chloroform (0.610%) and finally n-hexane (2.573%).

Our results showed that methanol was efficient in extracting phytochemicals more than other solvents. The yield of each extract was also different according to method of extraction and plant material.

Table 4: Percentage Yields of maceration and Soxhlet using different extraction solvents of five Sudanese Medicinal Plants:

| NO. | Scientific name       | Part Used | Percentage Yield (% w/w) | Maceration method | Soxhlet extraction method |
|-----|-----------------------|-----------|--------------------------|-------------------|---------------------------|
|     |                       |           |                          | n-hexane | Chloroform | Methanol | n-hexane | Chloroform | Methanol |
| 1   | *Euphorbia aegyptiacus* | WP        | 1.923                    | 1.139    | 7.123      | 3.150    | 0.763    | 6.137      |         |
| 2   | *Euphorbia acaephyoides* | WP       | 1.850                    | 2.164    | 5.097      | 2.782    | 1.359    | 8.456      |         |
| 3   | *Francoeuria crispa*   | WP        | 0.711                    | 1.410    | 5.610      | 1.458    | 1.410    | 8.455      |         |
| 4   | *Grewia tenax.*        | R         | 0.318                    | 0.315    | 1.814      | 0.670    | 0.217    | 3.623      |         |
| 5   | *Cissus quadrangularis* | WP       | 1.201                    | 1.154    | 12.189     | 2.573    | 0.610    | 5.464      |         |

Key: WP= Whole Plant, R= Roots.

Antioxidant activity

**DPPH radical scavenging assay**

The effect of two extraction techniques on antioxidant activity of the extracts was investigated. Maceration and Soxhlet successive extraction with n-hexane, chloroform and methanol were used. The DPPH radical scavenging activities of different plant extracts has been reported in Table 5 and 6.
The qualitative and quantitative determination of these patent antioxidants in the methanolic extracts of the selected Sudanese plants based on successive Soxhlet extraction could lead to isolation and structure determination of new naturally occurring potential antioxidants.

Table 5: DPPH radical scavenging activity of maceration method and soxhlet extraction method using different extraction solvents of five Sudanese Medicinal Plants.

| NO. | Scientific name       | %RSA ±SD (DPPH) | Maceration method | Soxhlet extraction method |
|-----|-----------------------|-----------------|-------------------|---------------------------|
|     |                       |                 | n-hexane          | Chloroform | Methanol | n-hexane | Chloroform | Methanol |
| 1   | Euphorbia aegyptiaca  | 3±0.04          | 7±0.08            | 96±0.01     | Inactive | 26±0.04 | 92±0.01 |
| 2   | Euphorbia acalyphoides| 13±0.09         | 15±0.01           | 85±0.05     | 5±0.06  | 7±0.05  | 82±0.01 |
| 3   | Francoeuria crispa    | 10±0.08         | 30±0.09           | 85±0.03     | 8±0.05  | 49±0.05 | 87±0.01 |
| 4   | Grewia tenax.        | Inactive        | 26±0.02           | 77±0.04     | 10±0.05 | 56±0.02 | 69±0.06 |
| 5   | Cissus quadrangularis | 3±0.07          | 9±0.06            | 69±0.01     | 4±0.02  | 30±0.05 | 75±0.04 |
|     | Propyl Gallate       |                 | 91±0.01           |            |         |         |         |

The results are presented as mean ± SEM. Each experiment was repeated three times; (n =3)

Key: RSA= Radicals scavenging activity, DPPH= 1,1-diphenyl-2-picrylhydrazyl.
Control (PG) = Propyl Gallate.

Figure 2: DPPH radical scavenging activity of maceration method and soxhlet extraction method using different extraction solvents of five Sudanese Medicinal Plants.

Table 6: DPPH radical scavenging activity and IC50 Value in methanol extraction by using maceration and soxhlet extractions of five Sudanese Medicinal Plants.

| NO. | Scientific name       | Methanol extract | Soxhlet extraction method |
|-----|-----------------------|------------------|---------------------------|
|     |                       | %RSA ±SD (DPPH)  | IC50 ±SD µg/ml (DPPH) |
|     |                       |                  | %RSA ±SD (DPPH)  | IC50 ±SD µg/ml (DPPH) |
| 1   | Euphorbia aegyptiaca  | 96±0.01          | 0.011±0.01               | 92±0.01 | 0.033±0.01 |
| 2   | Euphorbia acalyphoides| 85±0.05          | 0.150±0.02               | 82±0.01 | 0.173±0.03 |
| 3   | Francoeuria crispa    | 85±0.03          | 0.153±0.03               | 87±0.01 | 0.181±0.02 |
| 4   | Grewia tenax.        | 77±0.04          | 0.207±0.04               | 69±0.06 | 0.286±0.03 |
| 5   | Cissus quadrangularis | 69±0.01          | 0.271±0.05               | 75±0.04 | 0.229±0.03 |
|     | Propyl Gallate       | 91±0.01          | 0.077±µg/ml±0.01         | 91±0.01 | 0.077±µg/ml±0.01 |

Key: IC50= half concentration of inhibition. The lower IC50 value indicates the greater overall effectiveness of the antioxidant.
CONCLUSION

Choices of extraction method and solvent play important roles on maximizing extract yield and bioactivity. A comparative study has been conducted to assess the antioxidant activity of the extracts prepared by two different extraction methods of five Sudanese Medicinal plants.

Our results showed that methanol was efficient in extracting phytochemicals more than other extraction solvents. The yield of each extract was also different in the two methods and Soxhlet extraction gave maximum yields. The methanol was the best extraction solvent, which showed the maximum antioxidant activity followed by chloroform and finally n-hexane.

The results of our study revealed that methanol extracts prepared by maceration technique, exhibited better antioxidant activities. It is concluded from the study that maceration technique is more effective as compared to Soxhlets techniques.

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