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Osman Adam Osman Adam
Department of Pharmacognosy, Faculty of Pharmacy, Al-Neelain University. Khartoum, Sudan

Ragaa Satti Mohmmed Abadi
Department of Chemistry, Faculty of Science and Technology, Al-Neelain University. Khartoum, Sudan

Saad Mohamed Hussein Ayoub
Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology. Khartoum, Sudan

Correspondence:
Osman Adam Osman Adam
Department of Pharmacognosy, Faculty of Pharmacy, Al-Neelain University. Khartoum, Sudan
Email: osmanadam51[at]yahoo.com

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

Osman Adam Osman Adam*, Ragaa Satti Mohmmed Abadi, Saad Mohamed Hussein Ayoub

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-hydrazyl) 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-tag*ar. 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 of 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* Boiss	Euphorbiaceae	*Um lebaina. (malbein)*	Whole Plant
2	*Euphorbia acalyphoides* Hochst.ex. Boiss	Euphorbiaceae	*Um lebaina. (labien)*	Whole Plant
3	*Francoeuria crispa* (forssk.) cass.	Asteraceae.	*Al-tagar.*	Whole Plant
4	*Grewia tenax*. (Forssk.) fiori,	Tiliaceae.	*Godhaim, guddaim*	Roots
5	*Cissus quadrangularis* L.	Vitaceae.	*Salala*	Whole Plant

Figure 1: Study site: White Nile state in Sudan.

Chemicals

n- Hexane, Scharlau, Spain. Chloroform LR, CDH, India. Methanol LR, SDFCL, India. Ethanol LR, Duksan, South Korea. Dimethyl sulfoxide (DMSO), SDFCL, India. Propyl Gallate, Scott Science, UK. 2.2Di (4-tert-octylphenyl)-1-picryl-hydrazyl (DPPH), Sigma-Aldrich, UK.

Extraction of plant material

Maceration Method

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.

Soxhlet extraction 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.

$$\%\text{Yield of extract (g/100 g)} = (W_1 \times 100). / W_2$$

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-picryl-hydrazyl) stable free radical for half an hour at 37ºC. 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: 517nm 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.

Table 2: Conditions used to compare maceration and Soxhlet extractions

NO.	Parameter	Maceration extraction	Soxhlet extraction
1	Sample size (g)	30g	30g
2	Extraction solvent	n- Hexane, chloroform and methanol.	n- Hexane, chloroform and methanol.
3	Solvent volume (ml)	400 ml	400 ml
4	Temperature (°C)	Room Temperature (37 °C)	n- Hexane (69 ^{0}C), chloroform (61 ^{0}C) and methanol (65 ^{0}C).
5	Time	72h	24h

Table 3: Physicochemical Properties of Solvents (n-hexane, Chloroform and Methanol) Used in extraction [20].

No.	Solvent	Polarity index	Boiling point (^{0}C)	Viscosity (cPoise)	Solubility in water (% w/w)
1	n-hexane	0.0	69	0.33	0.001
2	Chloroform	4.1	61	0.57	0.815
3	Methanol	5.1	65	0.60	100

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 aegyptiaca* 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. acalyphoides* 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 aegyptiaca*	WP	1.923	1.139	7.123	3.150	0.763	6.137
2	*Euphorbia acalyphoides*	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 methanol extracts prepared by maceration or Soxhlet extraction with solvents of increasing polarity gave the best results. The type of the antioxidant secondary metabolites in the methanolic extracts of the five selected plants affected their activity. It was reported that tannins, flavonoids, coumarins, triterepenes and sterols were detected in these plants [21]. The quantities of these metabolites and their ratios in the said plants could give solid grounds to support our findings bearing on mind that they were reported as potent natural antioxidants [22, 23].

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-picryl hydrazyl.
Control (PG) = Propyl Gallate.

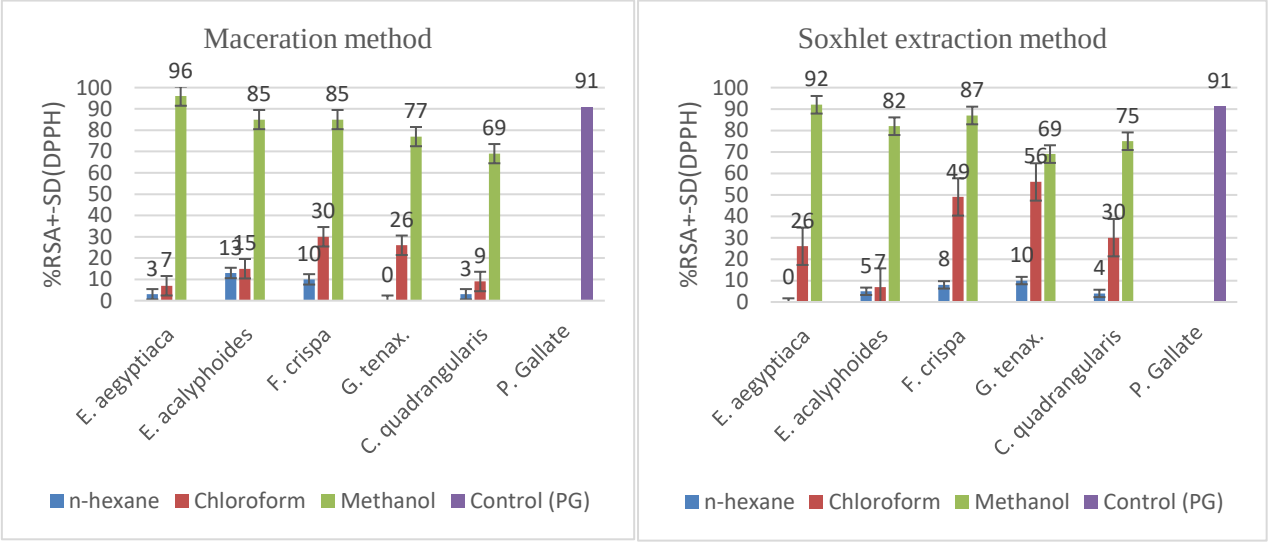

Figure 2: DPPH radical scavenging activity of maceration method and soxhlet extraction method using different extraction solvents of five Sudanese Medicinal Plants.

Table 6: DPPH radical scavenging activity and IC_{50} Value in methanol extraction by using maceration and soxhlet extractions of five Sudanese Medicinal Plants.

NO.	Scientific name	Methanol extract			
		Maceration method		Soxhlet extraction method	
		%RSA ±SD (DPPH)	IC_{50} ±SD µg /ml (DPPH)	%RSA ±SD (DPPH)	IC_{50} ±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	0.077µg/ml± 0.01	91±0.01	0.077µg/ml± 0.01

Key: IC_{50}= half concentration of inhibition. The lower IC_{50} 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 techniques, exhibited better antioxidant activities. It is concluded from the study that maceration technique is more effective as compared to Soxhlets techniques.

REFERENCES

1. Halliwell B. Biochemistry of Oxidative Stress," Bioche- mical Society Transactions, 2007; 35(5):1147-1150.
2. Sini KR, Sinha BN, Karpagavalli M. Determining the antioxidant activity of certain medicinal plants of Attapady, (Palakkad), India using DPPH assay. Current Botany 2010; 1(13-16):2220-4822.
3. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol. 2001; 54:176- 186.
4. Chanda S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiological Research. 2009; 3:981-996.
5. Marium A. Abo-dola, Mohamed F. Lutfi. Anti-inflammatory activity of *Euphorbia aegyptiaca* extract in rats. International Journal of Health Sciences. Qassim University. 2016; 10:1.
6. Liu ZG, Li ZL, Bai J, Meng DL, Li N, Pei YH, *et al*. Anti-inflammatory diterpenoids from the roots of Euphorbia ebracteolata. J Nat Prod. 2014; 25; 77(4):792-9.
7. Llanes-Coronel DS, Gámez-Díaz LY, Suarez-Quintero LP, Páez LJ, Torres F, Echeverri F, *et al*. New promising Euphorbiaceae extracts with activity in human lymphocytes from primary cell cultures. Immunopharmacol Immunotoxicol. 2011; 33(2):279-90.
8. Stavri M, Mathew KT, Gordon A, Shnyder SD, Falconer RA, Gibbons S. Guaianolide sesquiterpenes from Pulicaria Crispa (Forssk.) Oliv. Phytochemistry. 2008; 69:1915-1918.
9. Khemiss F, Ghoul-Mazgar S, Moshtaghie A, Saidane D. Study of the effect of aqueous extract of Grewia tenax fruit on iron absorption by everted gut sac. J. Ethnopharmacol. 2006; 103:90-98.
10. El- Kamali H, El- Khalifa K. Folk medicinal plants of riverside forests of the Southern Blue Nile district, Sudan. Fitoterapia. 1999; 70:493- 497.
11. Kumar S, Parveen F, Goyal S, Chauhan A. Indigenous herbal coolants for combating heat stress in the hot Indian arid zone. Ind. J. Tradit. Knowl. 2008; 7:679-682.
12. Al-Said MS, Mothana RA, Al-Sohaibani MO, Rafatullah S. Amelio rative effect of Grewia tenax (Forssk) Fiori fruit extract on CCl4–induced oxidative stress and hepatotoxicity in rats. J. Food Sci. 2011; 76:T200-T206.
13. Mishra G, Srivastava S, Nagori BP. Pharmacological and therapeutic activity of Cissus quadrangularis: an overview. Inter J Pharm Tech Res. 2010; 2:1298-1310.
14. Oben J, Kuate D, Agbor G, Momo C, Talla X. The use of a Cissus quadrangularis formulation in the management of weight loss and metabolic syndrome. Lipids Health Dis. 2006; 5:24.
15. Murthy KNC, Vanitha A, Swamy MM, Ravishankar GA. Antioxidant and antimicrobial activity of *Cissus quadrangularis* L. J Med Food. 2003; 6:99-105.
16. Chopra SS, Patel MR, Awadhiya RP. Studies of *Cissus quadrangularis* in experimental fracture repair: a histopathological study. Indian Journal of Medical Research. 1976; 64:1365-1368.
17. Shirwaikar A, Khan S, Malini S. Antiosteoporosis effect of ethanol extract of *Cissus quadrangularis* Linn. On ovariectomized rat. Journal of Ethonopharmacology. 2003; 89:245-250.
18. Attawish A, Chavalttumrong D, Chivapat S, Chuthaputti S, Rattarajarasroj S, Punyamong S. Subchronic toxicity of *Cissus quadrangularis* Linn. Songklanakarin J Sci Tech. 2003; 24:39-51.
19. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidant properties of xanthan on the abutoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural Food Chemistry. 1992; 40:945-948.
20. Satyajit D Sarker, Lutfun Nahar. Natural Products Isolation. 3rd ed. Springer Nature: New York, 2012; 30:31.
21. Osman Adam Osman Adam, Ragaa Satti Mohmmed Abadi, Saad Mohamed Hussein Ayoub. Chemical constituents and antioxidant activity of some Sudanese medicinal plants. Journal of Pharmacognosy and Phytochemistry. 2018; 7(6):1751-1755.
22. Foti MC. Antioxidant properties of phenols, J Pharm Pharmacol. 2007; 59(12):1673-85.
23. Gan R-Y, Xu X-R, Song F-L, Kuang L, Li H-B. Antioxidant activity and total phenolic content of medicinal plants associated with prevention and treatment of cardiovascular and cerebrovascular diseases. Journal of Medicinal Plants Research. 2010; 4(22):2438-2444.

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