GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Phytochemical screening, TLC profile and antioxidant activity of hydromethanolic extract of *Centaurothamnus maximus* leaves

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GSC Biological and Pharmaceutical Sciences, 2023, 25(02), 230–239

Publication history: Received on 21 October 2023; revised on 14 November 2023; accepted on 17 November 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.25.2.0453

Abstract

The species *Centaurothamnus maximus* (Forssk.) Wagenitz & Dittrich is grown in different places in Yemen and is used to treat many diseases. The aim of this research was to investigate the phytochemicals and the antioxidant activity of hydromethanolic extract obtained from this plant. For preliminary phytochemical analysis, standard procedures were applied. The Folin–Ciocalteu method was used to evaluate the total phenolic acid content of the plant extract, while the total flavonoid content was determined using the aluminum chloride colorimetric assay. The antioxidant activities were assessed using the 1,1-diphenyl-2-picrylhydrazyl. Chemical analysis revealed the presence of carbohydrates, saponins, polyphenols, flavonoids, triterpenes, sterols, tannins and alkaloid. The total content of phenols was 43.04±3.30 mg gallic acid equivalent per gram dry extract, while the total flavonoid content was 36.60±2.86 mg rutin equivalent per gram dry extract. With TLC and specific reagents, flavonoid and phenolic substances were identified. The hydromethanolic extract and its fractions showed a dose dependent scavenging activity, we found that the ethyl acetate fraction has high inhibitory percentages equivalent to IC_{50} 40.86±0.001 for DPPH. These results indicate that the leaves of *Centaurothamnus maximus* are rich in secondary metabolites, and may be used as a natural anti-oxidant source. Extensive chemical and pharmacological studies should be carried out

Keywords: Yemen; *Centaurothamnus maximus*; Leaves; Phytochemicals; Antioxidant

1. Introduction

Medicinal plants are considered one of the most important natural sources for obtaining medicines and contribute greatly to the discovery of new medicinal compounds, despite the great scientific progress in the chemistry [1, 2]. Any medicinal substance must conform to standards of quality, safety, and efficacy. Therefore, pharmacognosy, which is a well-established pharmaceutical science, plays a diverse role in the discovery, characterization, production, and standardization of herbal drugs [1]. Sensory and the phytochemical screening methods are employed for identification and authentication of raw and final herbal medicines in order to detect the species-based specific compounds (such as flavonoids, triterpenes, alkaloids, saponins) by precipitation or colour reactions in raw plant materials, followed by TLC separation of extracts using suitable solvent systems and specific spray reagents [3, 4]. Many plants containing phytochemicals have curative/protective properties against various diseases. Most phytochemicals, especially phenolics have health benefits by scavenging free radicals or quenching reactive oxygen species [5, 6].

In general, antioxidants are defined as substances that are able to reduce oxidation and are able to maintain the oxidative balance, thus avoiding the ailments prevalence [7], and therefore interest in studying the antioxidant activity of medicinal plants is increasing [8, 9].

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Nowadays, a lot of research has appeared on medicinal plants in Yemen, which contain important scientific information. However, many of these plants have not been studied or studied little [10, 11, 12]. The plant *Centaurothamnus maximus* (Forssk) Wagenitz & Dittrich is one of the few studied plants despite it's widespread popular use in Yemeni traditional medicine [13]. In our previous study, the macro and microscopic characteristics, physicochemical parameters, and antibacterial activity of the *Centaurothamnus maximus* leaves were determined [12]. In addition, extracts from *Centaurothamnus maximus* showed cytotoxic activities against several tumour cell lines [13]. The aim of this study is to investigate the chemical composition and free radical scavenging activity of a hydromethanolic extract from the leaves of *Centaurothamnus maximus* and correlate the scientific basis of their medicinal use with the traditional medicine of Yemen.

2. Material and methods

2.1. Collection and identification of plant material

Fresh leaves of *Centaurothamnus maximus* were collected in May 2019 in Yafa- the Republic of Yemen, dried in the shaded area, then manually ground and stored at room temperature for further analysis. The plant sample was identified by a taxonomist, Associate Professor Othman S. Alhawshibi, of the faculty of science, University of Aden, Yemen.

2.2. Preparation of the extract

The dried powdered leaves (50 gm) of *Centaurothamnus maximus* were defatted with petroleum ether (boiling point 60-80 ℃) in Soxhlet extractor. The marc left after petroleum ether extraction was dried completely in hot air oven below 50 ℃ and then packed well in Soxhlet apparatus and extracted with 80% methanol (80-90 ℃), until the extraction was completed. The 80% methanolic extract was evaporated and concentrated to dryness using the rotary evaporator at 50 ℃ and the percentage yield in term of air dried material was calculated, then stored used for further analysis [14].

2.3. Fractionation of 80% methanol extract

Fractionation of 80% methanol extract was carried out with different organic solvents. A portion of the methanolic extract was suspended in water, extracted successively with chloroform, ethyl acetate and n butanol (3×300 ml each) and then resulting solutions were concentrated to provide chloroform, ethyl acetate, n butanol and water residue fractions [14].

2.4. Qualitative phytochemical analysis

Preliminary analysis of 80% methanol extract was carried out to identify the presence of various phytoconstituents by employing standard protocols [14, 15].

2.5. Assessment of total phenolic content (TPC)

To determine the total phenolic content of the *Centaurothamnus maximus* leaves extract using the Folin-Ciocalteu reagent, Ainsworth's method was significantly modified [16. 17]. The calibration curve's reference standard for plotting was gallic acid. The Folin-Ciocalteu reagent was diluted 1:10 with deionized water, and 0.5 mL of the plant extract (100 g/mL) was added. This mixture was then neutralized with 4 mL of sodium carbonate solution (7.5%, w/v). Over the duration of the 30-minute incubation period, the reaction mixture was shaken intermittently to promote color development. Using a double beam UV-VIS spectrophotometer, the absorbance of the resultant blue color was determined at 765 nm (UV Analyst-CT 8200). With a standard curve established with a linear equation, the total phenolic contents were calculated.

2.6. Assessment of total flavonoid content (TFC)

The aluminum chloride colorimetric technique was used to calculate the TFC of the 80% methanol extract [18]. Rutin was added in various doses (20-100 g/mL) together with 2 ml of distilled water and then 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution, and the mixture was then added. 0.15 ml of (10% $AlCl_3$, w/v) solution was added after 6 minutes. After another 6 minutes of standing time, 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. With immediate addition of distilled water, the final amount was changed to 5 ml, well mixed, and let to stand for an additional 15 minutes. At 513 nm, the absorbance of each combination was measured in comparison to the other mixtures.

2.7. Thin layer chromatography studies

Thin Layer Chromatography of prepared extracts was performed to determine Rf values. Various solvent systems were tested to obtain best results. The results of separation identified under UV light (254 nm and 366 nm). Furthermore, the TLC plate was sprayed with specific sprayers $FeCl_3$ and $AlCl_3$ then calculated value of Rf [19, 20].

2.8. DPPH free radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to assess the DPPH radical scavenging activity of 80% methanol extract, chloroform, ethyl acetate and n-butanol fractions of *Centaurothamnus maximus* leaves [21]. The standard rotin and samples were dissolved in methanol and prepared at 1mg/ml concentrations. Concentrations of 500, 350, 200, 150, 100, 50 µg/ml dilutions were made using methanol. Freshly prepared 3ml of 0.1mM solution of DPPH in methanol was mixed 1ml of each sample solution. The solutions were kept for 30 minutes at room temperature and the absorbance was determined at 517nm. The solutions were prepared in triplicates for the analysis and calculated the mean values of absorbance. The percentage of DPPH radical scavenging activity was determined using the following formula- (%) DPPH radical scavenging inhibition =

$$[(A0 - At/A0)] \times 100$$

Where A0 was control absorbance and At was sample absorbance. All the experiments were performed in triplicate and the graph was plotted with the mean ± SD values.

2.9. Calculation of IC_{50}

Inhibition Concentration (IC_{50}) parameter was used [22]. for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC_{50} value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50%.

2.10. Statistical analysis

Analysis of variance of data was evaluated by Student's t test P-values less than 0.05 was considered to be statistically significant.

3. Results and discussion

3.1. Qualitative Phytochemical Studies

Medicinal plants constitute the main source of new medicines and healthcare products [23]. Some of these plants are an important source of natural antioxidants that have been shown to reduce the risk and development of certain acute and chronic diseases such as cancer, heart disease, and stroke by scavenging free radicals. Moreover, the existence of secondary plant metabolites including alkaloids, flavonoids, glycosides, tannins, steroids, etc. might be attributed to the various pharmacological effects of medicinal plants [24, 25]. The extract of *Centaurothamnus maximus* leaves included many kinds of secondary metabolites, including carbohydrates, saponins, polyphenols, flavonoids, triterpenes, sterols, tannins, and alkaloids, according to the results of phytochemical screening (Table 1). The percentage yield of 80% methanolic extract of the leaf powder was 24 % and the color was brownish-green.

3.2. Determination of total phenolic content (TPC)

Phenolic compounds are excellent oxygen radical scavengers [10, 11]. Numerous techniques for determining the total phenolic content of foods or biological samples rely on the interaction of phenolic compounds with a colorimetric reagent, which enables assessment in the visible spectrum [26, 27]. The Folin–Ciocalteu (F–C) assay is such a method [28, 29]. The present study has been carried out for quantification of the total phenolic content of methanol 80% extract of *Centaurothamnus maximus* leaves. The content of the phenolic compounds in the selected leaves extract, determined from regression equation of calibration curve (y=0.0152x–0.0412, R2 = 0.9844) of Gallic acid (20–100 µg/mL) and expressed in mg gallic acid equivalent (GAE) per gram dry extract. The result of total phenolic content (TPC) was 43.04±3.30 mg/g of plant extract. The standard calibration curve of gallic acid is shown in Figure 1.

Table 1 Results of phytochemical screenings of 80% methanolic extract of the leaves of *Centaurothamnus maximus*

Phytochemical Screening		**Methanol Extracts**
Carbohydrates	Fehling's test	+++
	Molisch's test	++
Amino acids/protein	Ninhhydrin test	-
Sterols/Triterpenes	Salkowski test	+++
	Liebermann-Burchard test	+++
Alkaloids	Wagner's test	++
	Mayer's test	+
	Dragendroff's test	++
Polyphenols	Ferric chloride test	+++
Flavonoids	Shinoda test	+++
	NaOH Test	+++
	Lead acetate test	+++
	Aluminium solution test	+++
Tannins	Ferric chloride test	+++
Saponins	Foam test	+++

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

3.3. Determination of total flavonoid content (TFC)

Currently, many of the health-related effects of flavonoids are attributed to their antioxidant properties, whereas a wide variety of products containing them are commercially available [30, 31]. The total flavonoids content of selected leaves extract was determined from regression equation of calibration curve (y=0.0063x+0.097, R2= 0.9971) of rutin (20-100 µg/mL) and expressed in mg rutin equivalent (RE) per gram dry extract. The result of total flavonoid content (TFC) was 36.60±2.86 mg/g of plant extract. The standard calibration curve of rutin is shown in Figure 2.

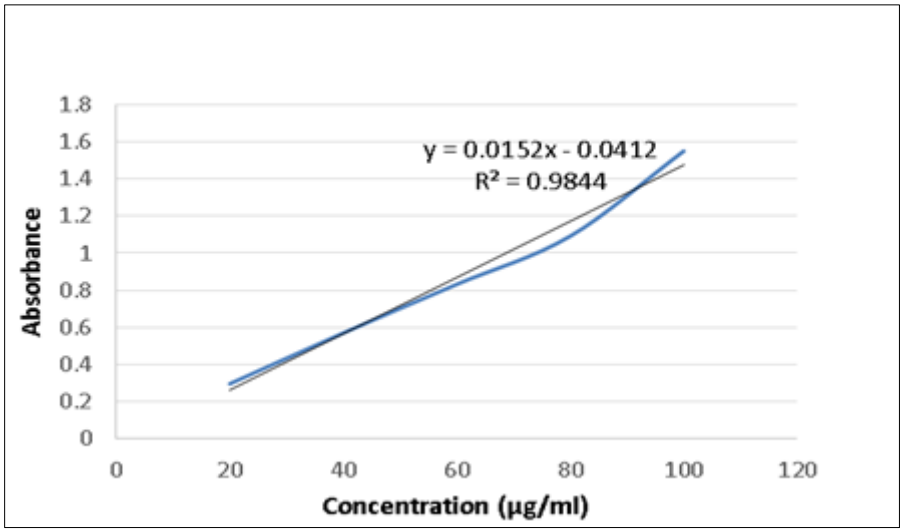

Figure 1 Standard calibration curve of gallic acid

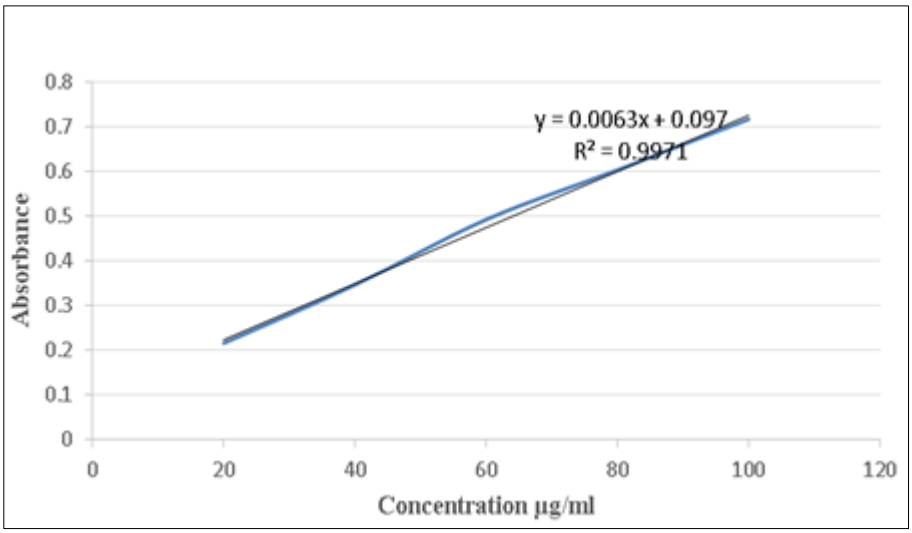

Figure 2 Standard calibration curve of rotin

3.4. Thin layer chromatography

The presence of phytoconstituents was further confirmed by thin layer chromatography. The solvent system chloroform-methanol-formic acid (44:3.5:2.5) was the best system used for spot separation in the 80% methanol extract and it's fractions. The plates were sprayed with 5% $FeCl_3$ reagents that identified flavonoid and polyphenol compounds and will turn to brown, grey and black colors, indicating flavonoids and polyphenols content in extracts [32]. The results of the detection with $FeCl_3$ spray showed the present of phenol compounds. Detection of compounds with the spray reagent 10% $AlCl_3$ followed the present of flavonoids. Results were shown in Table 2 and Figures 3 and 4.

Table 2 Observations of TLC of extracts of *Centaurothamnus maximus* leaves in chloroform-methanol-formic acid (44:3.5:2.5)

Extract/fraction	No. of spots	Rf values	Color of spots			
			at 254 nm	at 365 nm	with $FeCl_3$	with $AlCl_3$
80% Methanolic extract	13	0.18	-	Sky blue	-	-
		0.20	-	Sky blue	-	-
		0.25	-	Blue	-	-
		0.31	-	Light blue	-	-
		0.40	Dark	Deep violet	Dark blue	Yellow
		0.42	Light dark	Brownish blue	-	-
		0.45	Dark	Purple	Light blue	Light yellow
		0.51	-	Light brown	Light blue	-
		0.53	-	Pink	-	-
		0.56	Dark	Sky blue	-	-
		0.77	-	Sky blue	-	-
		0.80	-	Red	-	-
		0.87	Light dark	Deep red	-	-
Chloroform fraction	14	0.18	-	Light blue	-	-
		0.41	Light dark	Purple	-	-

		0.43	Light dark	Light pink	-	-
		0.51	Light dark	Purple	-	-
		0.53	-	Light pink	Light blue	Light yellow
		0.54	Light dark	Purple	Dark blue	Light yellow
		0.55	-	Pink	Light blue	Light yellow
		0.56	Dark	Sky blue	-	-
		0.59	-	Pink	-	-
		0.64	Light dark	Light red	-	-
		0.60	-	Light pink	-	-
		0.76	Light dark	Light blue	-	-
		0.81	-	Deep red	-	-
		0.87	Light dark	Deep red	-	-
Ethyl acetate fraction	8	0.18	-	Blue	-	-
		0.20	Light dark	Light violet	-	-
		0.23	Light dark	Blue	-	Yellow
		0.35	Deep dark	Deep violet	Light blue	Deep yellow
		0.42	-	Blue	Light blue	Deep yellow
		0.45	Deep dark	Deep violet	Deep blue	Yellow
		0.51	Light dark	Light blue	Light blue	-
		0.57	Light dark	Deep sky blue	-	-
		0.86	Light dark	Blue	-	-
		0.87	-	Pink	-	-
n-Butanol fraction	5	0.18	-	Sky blue	-	-
		0.24	-	Sky blue	-	-
		0.30	-	Light blue	-	-
		0.40	Light dark	Violet	Light blue	-
		0.42	Light dark	Light blue	-	-

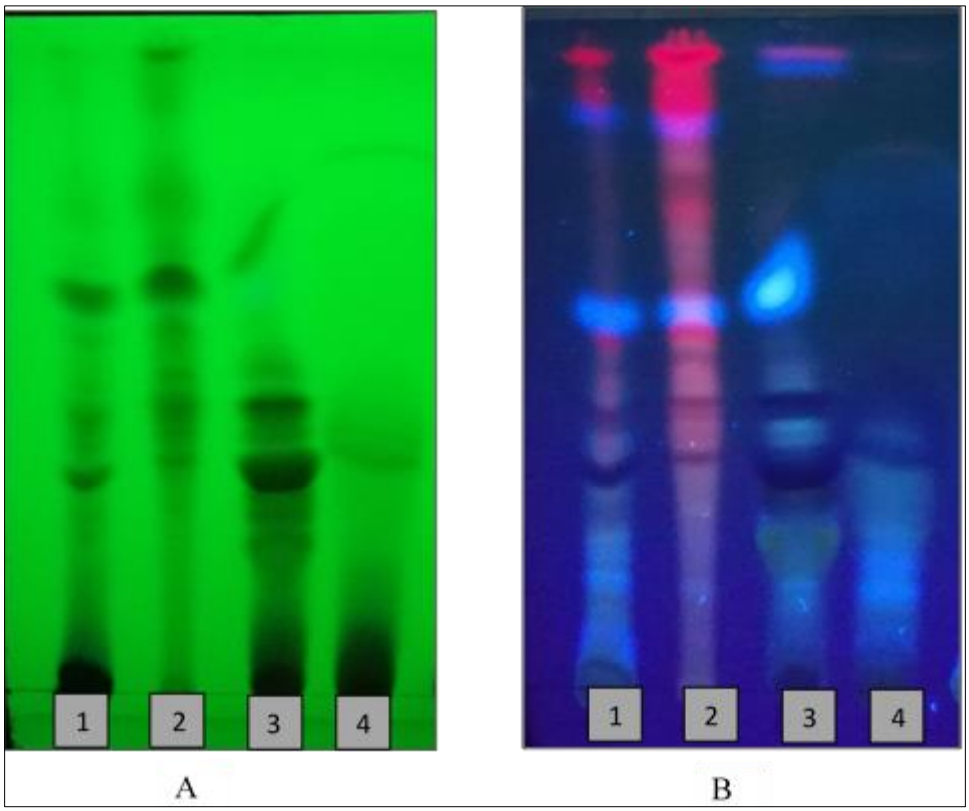

Figure 3 TLC plates of 80% methanolic extract (1), chloroform (2), ethyl acetate (3) and n-butanol (4) fractions obtained in chloroform-methanol-formic acid (44:3.5:2.5) under UV 254 nm (A), UV 365nm (B)

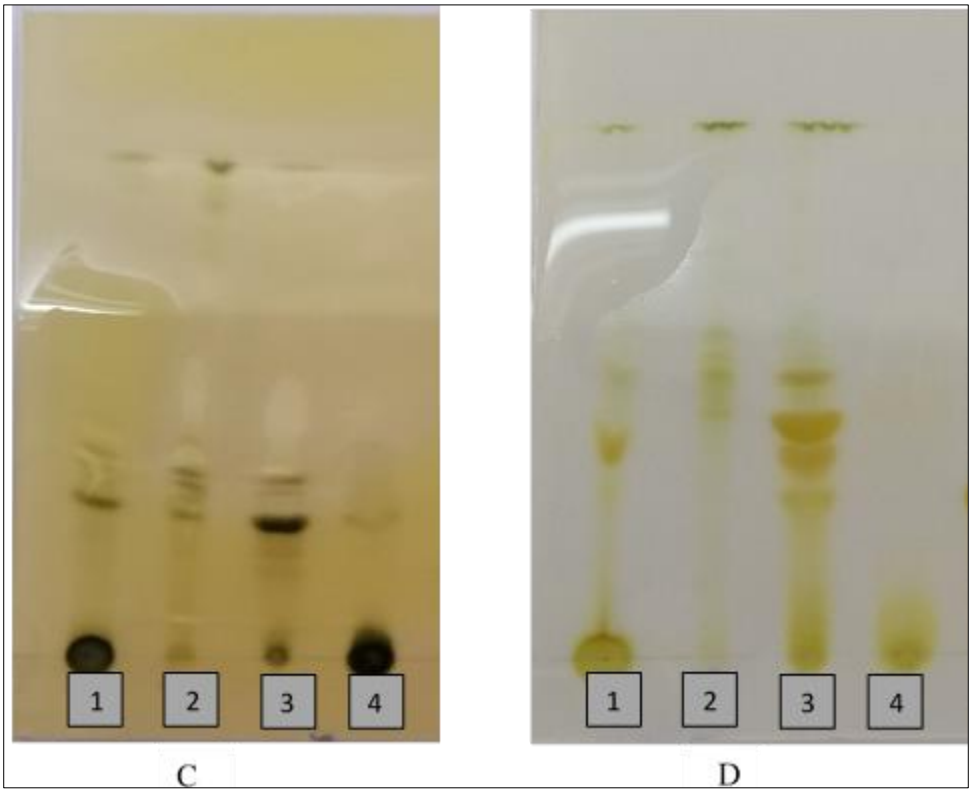

Figure 4 TLC plates of 80% methanolic extract (1), chloroform (2), ethyl acetate (3) and n-butanol (4) fractions obtained in chloroform-methanol-formic acid (44:3.5:2.5) in day light after staining with $FeCl_3$ (C) and with $AlCl_3$ (D)

3.5. DPPH free radical scavenging activity

An accessible and popular method for evaluating the in-vitro antioxidant activity of natural substances or plant extracts is DPPH free radical scavenging activity [33, 34]. The scavenging effect of different concentration of extract and fractions of *Centaurothamnus maximus* leaves on the DPPH free radical was compared with the standard anti-oxidant rotin. The results were expressed as inhibition (%) shown in Table 3 and Figure 5. The hydromethanolic extract and its fractions showed a dose dependent scavenging activity. However, their scavenging ability was found to be significant (P >0.05) in comparison to rotin.

Table 3 DPPH free radical scavenging activity of rotin, extract and fractions of *Centaurothamnus maximus* leaves

Concentrations µg/ml	**Radical scavenging effect (%)**				
	Rotin	**Methanol 80% extract**	**Chloroform fraction**	**Ethyl acetate fraction**	**n-Butanol fraction**
50	56.00±2.22	19.71±4.22	16.26±3.23	45.96±3.23	24.91±3.38
100	71.00±4.12	37.14±1.24	28.67±2.41	60.60±4.01	43.61±4.16
150	80.60±3.25	46.96±5.01	37.84±3.32	76.54±3.22	61.06±3.11
200	90.10±5.12	62.88±2.11	46.84±3.33	84.30±2.44	70.31±2.12
250	95.10±4.32	70.81±3.22	62.84±3.33	86.54±4.13	76.84±3.43
IC_{50}, µg/ml	3.23	159.75	201.65	40.86	129.53

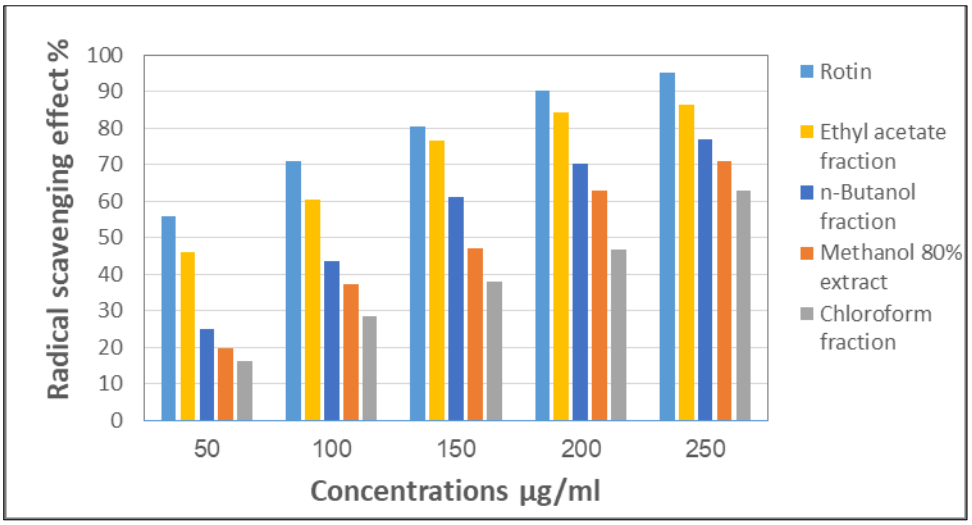

Figure 5 DPPH free radical scavenging activity of rotin, extract and fractions of *Centaurothamnus maximus* leaves

4. Conclusion

In this study on the *Centaurothamnus maximus* leaves, important chemicals such as carbohydrates, saponins, polyphenols, flavonoids, triterpenes, sterols, tannins and alkaloid were identified in the hydromethanolic extract, also the hydromethanolic extract and its fractions showed significant inhibition effect on DPPH. These results confirm the traditional use of the plant and are the basis for further chemical and therapeutic studies.

Compliance with ethical standards

Disclosure of conflict of interest

The manuscript has no conflict of interest.

References

[1] Peter J Houghton. The role of plants in traditional medicine and current therapy. J. Altern. Complement. Med. 1995; 1(2): 131-143.

[2] Muela SH, Ribera JM, Mushi AK, Tanner M. Medical syncretism with reference to malaria in a Tanzanian community. Soc. Sci. Med. 2002; 55(3): 403-413.

[3] Mouhssen Lahlou. Screening of natural products for drug discovery. Expert Opin. Drug Discov. 2007; 2(5):697-705.

[4] Patel PM, Patel NM, Goyal RK. Quality control of herbal products. The Indian Pharmacist. 2006; 5(45):26-30.

[5] Halliwell B. Antioxidants and human disease: a general introduction. Nutr. Rev. 1997; 55(1): 44-49.

[6] Tsai PJ, Wu SC, Cheng YK. Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. Food Chem. 2008; 106 (1): 27-32

[7] Lourenço SC; Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. Molecules. 2019; 24(22): 4132-4156 [CrossRef]

[8] Swallah MS, Sun H, Affoh R, Fu H, Yu H. Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. Int. J. Food Sci. 2020; 2020: 9081686. 8 p.

[9] Shetty A, Magadum S, Managanvi K. Vegetables as sources of antioxidants. J. Food Nutr. Disord. 2013; 2(1): 1–5. [Google Scholar] [CrossRef]

[10] Al-Dubai AS, Al-khulaidi AA. Medicinal and Aromatic Plants of Yemen (In Arabic). Sana'a, Yemen: Obadi Center for Studies and Publishing; 1996.

[11] wadh NA, Juelich W-D, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J Ethnopharmacol. 2001; 74(2): 173–179.

[12] Algfri SK, Qaid AA, Naser Gulia A. Pharmacognostic and Antibacterial Activity of the *Centaurothamnus* maximus leaves. J. Med. Plants. Stud. 2022; 10(1): 107-110. DOI: 10.22271/plants.2022.v10.i1b.1368

[13] Ingrid Hehmeyer, Hanne Schönig. Herbal medicine in Yemen: Traditional Knowledge and Practice, and Their Value for Today's World, Edited by Ingrid Hehmeyer and Hanne Schönig with the collaboration of Anne Regourd, (Islamic history and civilization, v. 96). Boston: Brill, Leiden; 2012.

[14] Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edition. London: Chapman and Hall; 2007, 125-175.

[15] Evans WC. Trease and Evans Pharmacognosy. 15th edition, Sydney, Toronto: WB Saunders; 2002; 214–252.

[16] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc. 2007; 2(4):875-877.

[17] Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of Moringa oleifera. Asian Pac J Trop Biomed 2013; 3(8):623-627.

[18] Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64(4):555-559.

[19] Waksmundzka-Hajnos M, Sherma J, Kowalska T. Thin layer chromatography in phytochemistry. CRC Press; 2008.

[20] Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd edition. Berlin, Heidelberg, New York: Springer-Verlag; 1996.

[21] Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity in vitro of the selenium-contained protein from the Se-enriched Bifidobacterium animalis 01. Anaerobe. 2010; 16(4):380–386.

[22] Sase S, Limaye RP, Soni N, Gaikwad S. To study the action of Basella alba ethanolic extract on Calcium Oxalate in vitro. International journal of ayurvedic & herbal medicine. 2013; 3 (5): 1343-1346.

[23] Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology. 2005; 97(1-2): 145-150.

[24] Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahu A. Indian medicinal herbs as sources of antioxidants. Food Res Int. 2008; 41(1): 1-15.

[25] Pham-Huy LA, He H, Huyc CP. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008; 4(2): 89-96.

[26] Robards K, Antolovich M. Analytical chemistry of fruit bioflavonoids a review. Analyst. 1997; 122(2): 11R–34R.

[27] Magalhaes LM, Segundo MA, Reis S, Lima JL, Rangel AO. Automatic method for the determination of Folin–Ciocalteu reducing capacity in food products. J. Agric. Food Chem. 2006; 54(15): 5241–5246.

[28] Folin O, Ciocalteu V. On tyrosine and tryptophane determinations in proteins. J. Biol. Chem. 1927; 73(1,2): 627–650.

[29] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965; 16: 144–158.

[30] Benavente-García O, castillo J, Marin F, Ortunõ A, Del Rio JA. Uses and properties of citrus flavonoids. J. Agric. Food chem. 1997; 45: 4505.

[31] Havsteen BH. The biochemistry and medical signifcance of the flavonoids. Pharmacology & therapeutics. 2002; (2-3):67-202.

[32] Muflihah CH, Haryoto H, Indrayudha P. Cytotoxic assay of semipolar fraction of ethanolic extract from sugar apple (Annona squamosa l.) stem bark on T47D cells. Pharmacon J Farmasi Indones. 2020; 17(2):148–56. doi:10.23917/pharmacon.v17i2.12268

[33] Philips A, Philips S, Arul V, Padmakeerthiga B, Renju V, Santha S. Free radical scavenging activity of leaf extracts of Indigofera aspalathoides - An in vitro analysis. J Pharm Sci Res. 2010; 2: 322-328.

[34] Kusznierewicz B, Piekarska A, Mrugalska B, Konieczka P, Namieśnik J, Bartoszek A. Phenolic composition and antioxidant properties of polish blue-berried honeysuckle genotypes by HPLC-DAD-MS, HPLC postcolumn derivatization with ABTS or FC, and TLC with DPPH visualization. J. Agric. Food Chem. 2012; 7:1755-1763.