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Evaluation of the *in vitro* anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine

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The present research study deals with the evaluation of sixty four methanolic and aqueous extracts of thirty Yemeni plants used in traditional medicine for their *in vitro* antiproliferative activity against three human cancer cell lines in a microtiter plate assay based on cellular staining with crystal violet, for their antimicrobial activity against antibiotic susceptible three Gram-positive, three Gram-negative bacterial and one fungal stains and three multiresistant *Staphylococcus* strains by the agar diffusion method and the determination of MIC against three Gram-positive bacteria with the broth micro-dilution assay, as well as for their antioxidant activity using the DPPH radical scavenging method. Furthermore the chemical composition of the methanolic extracts was determined by using chromatographic methods. As a result of this work, 12 Yemeni herbs namely *Centaurothamus maximus*, *Costus arabicus*, *Cupressus sempervirens*, *Dichrocephala integrifolia*, *Euphorbia schimperi*, *Gomphocarpus fruticosus*, *Kanahia laniflora*, *Meriandera benghalensis*, *Pulicaria inuloides*, *Solanum glabratum*, *Tarconanthus camphoratus* and *Vernonia leopoldii* demonstrated a noteworthy growth inhibitory effect against all cancer cell lines with IC_{50} values < 50 μg/ml. Pronounced antimicrobial activity was observed only against Gram-positive bacteria among them multiresistant bacteria with inhibition zones > 15 mm and MIC values < 500 μg/ml, by 9 plants especially *Centaurothamus maximus*, *Cupressus sempervirens*, *Enicostemma verticillare*, *Meriandera benghalensis*, *Nepeta deflersiana*, *Pulicaria inuloides*, *Tarconanthus camphoratus*, *Teucrium yemense* and *Vernonia leopoldii*. Moreover, the methanolic extracts of *Cupressus sempervirens*, *Meriandera benghalensis*, *Pulicaria inuloides* and *Rhus retinorrhaea* showed a remarkable radical scavenging effect at low concentrations.

1. Introduction

Over the past decades, interest in drugs derived from higher plants and utilization of medicinal plants in the world, especially in developing countries, has increased markedly and contributes to primary health care in the third world. Many plants are used in the form of powders, crude extracts or infusions to treat several diseases including cancers as well as common viral and microbial infections without any scientific evidence of efficacy.

Hartwell (1982) listed more than 3000 plant species that have reportedly been used in the treatment of cancer, but in many instances, the "cancer" is undefined, or reference is made to conditions such as hard swellings, abscesses, calluses, corns, warts, polyps, or tumors, to name a few. Plants have served as important sources of effective anticancer agents and over 60% currently used anticancer agents were isolated from natural sources, including plants, marine organisms and microorganisms or are related to them (Cragg et al. 2005; Newman et al. 2003). These include the naturally derived taxanes e.g. paclitaxel isolated from *Taxus baccata* and *Taxus brevifolia*, etoposide and teniposide, the semi-synthetic derivatives of epipodophyllotoxin, isolated from species of the genus *Podophyllum*, the Vinca alkaloids, vinblastine and vincristine, isolated from *Catharanthus roseus*, the semisynthetic derivatives of camptothecin, irinotecan and topotecan, isolated from *Camptotheca acuminata*, and several others (Cragg and Newman 2000; Wang 1998). Thus the interest in the use of folk medicine for tumor treatment or prevention has increased (Mothana et al. 2007; Mosaddik et al. 2004).

In recent years, interest in plants with antimicrobial properties has been renewed since multiple drug resistance has developed in bacteria due to the unselective and careless use of commercial antimicrobial drugs (Okeke et al. 2005). The increasing prevalence of multi-resistant bacteria demands new antimicrobial compounds from various sources such as medicinal plants. Thus, the search for herb extracts may offer a unique potential for the development of novel agents that can combat otherwise difficult to treat infections (Cowan 1999).

It is already known that free radicals (ROS) are able to damage cellular constituents as DNA, proteins and lipids and act as secondary messenger in inflammation. Antioxidants can scavenge ROS and can also reduce inflammation pathways. The use of antioxidants may be useful in the treatment or prophylaxis of certain diseases, e.g. Alzheimer's disease, heart disease, stroke, arteriosclerosis, diabetes and cancers (Calabrese et al. 2003; Gibson and Huang 2005). Thus, there is much interest in identifying natural antioxidants.

Consequently, most of the research performed today focuses on the development of new drugs to treat cancer, as well as viral and microbial infections. The present study is a part of our ongoing studies on the biological activities of Yemeni medicinal herbs and aims to provide data on the growth inhibitory activities on three human cancer cell lines derived from different tissues, on the antimicrobial activities against several antibiotic susceptible and multiresistant bacterial and fungal strains as well as on the antioxidant potential of 64 extracts from 30 Yemeni medicinal plants.

2. Investigations, results and discussion

During our screening program for plants with *in vitro* antiproliferative, antimicrobial and antioxidant activities, different governorates in Yemen were visited, many elderly people with knowledge of folk medicine were interviewed and finally 30 plants belonging to 18 families were collected for the screening. A total of 64 methanolic and aqueous extracts were prepared and evaluated for their cytotoxic, antimicrobial and antioxidant activities. The botanical names, herbarium numbers, plant organ used and the traditional uses of the plants in the collected locations are demonstrated in Table 1. To estimate *in vitro* anticancer activity against three human cancer cell lines namely one lung cancer (A-427), one urinary bladder cancer (5637) and one breast cancer (MCF-7) cell line, a microtiter plate assay based on cellular staining with crystal violet was used. Table 2 reports the IC_{50} values for the antiproliferative activities of the investigated 32 methanolic extracts. It was demonstrated that 12 methanolic extracts out of 32 exhibited a noticable cytotoxic effect against all three tested cancer cell lines at concentrations <50 μg/ml. The most interesting results were obtained with *Gomphocarpus fruticosus*, *Kanahia laniflora*, *Meriandera benghalensis*

Table 1: List of plants screened

Plant	Voucher specimen no.	Family	Part tested	Traditional uses[a]
Acalypha ciliata Forssk.	Mo-I12	Euphorbiaceae	L	Anthelmintic and for scabies and malaria (3, 4)
Ajuga bracteosa Wall. ex Benth.	Mo-I10	Labiatae	L, F	Antiseptic and for teeth pains (4)
Barleria trispinosa (Forssk.) Vahl	Mo-M03	Achanthaceae	L, S	For warts epically on the face (3, 4)
Blepharis ciliaris (L.) B.L.Burtt.	Mo-H05	Achanthaceae	L	For wounds and skin diseases (4)
Buddleja polystachya Fresen.	Mo-M12	Loganiaceae	L, F	For leprosy, vitiligo and warts (4)
Centaurothamus maximus (Forssk.) Wagenitz & Dittrich	Mo-I07	Compositae	L, F	For wounds (4)
Chenopodium ambrosioides L.	Mo-S22	Chenopodiaceae	L	Anthelmintic, for skin and kidney diseases (2, 4)
Coccinia grandis (L.) Voigt	Mo-T07	Cucurbitaceae	L, T	Anthelmintic, diuretic and for pneumonia (4)
Costus arabicus L.	Mo-S05	Zingiberaceae	R	For cancers (4)
Cupressus sempervirens L.	Mo-S25	Cupressaceae	L	Expectorant, astringent and for wounds, diarrhea and hemorrhides (2, 4)
Dichrocephala integrifolia (L.f.) O. Kuntze	Mo-M04	Compositae	L, F	For wounds (4)
Dorstenia barnimiana Schweinf.	Mo-T09	Moraceae	L	For fungal diseases (4)
Enicostemma verticillare (Retz.) Baill.	Mo-I06	Gentianaceae	L	For diabetes (4)
Euphorbia schimperi Presl	Mo-T11	Euphorbiaceae	L	Antiseptic and for warts (1, 2, 4)
Gomphocarpus fruticosus (L.) Ait. f.	Mo-S23	Asclepiadaceae	L	For heart diseases (2, 4)
Grewia erythraea Schweinf.	Mo-S07	Tiliaceae	L, S	Diuretic and for kidney diseases and hemostatic(4)
Kanahia laniflora (Forsk.) R. Br.	Mo-I19	Asclepiadaceae	R	For tumors and skin diseases, scabies and itching (3, 4)
Kniphofia sumarae Deflers	Mo-I10	Liliaceae	L, F	For malaria (4)
*Lavandula dentat*a L.	Mo-I11	Labiatae	L, F	For wounds and rheumatism (2, 3, 4)
Leucas inflata Benth.	Mo-I05	Labiatae	L, F	For kidney diseases and tooth ache (4)
Lindenbergia indica (L.) Kuntze	Mo-S18	Scrophulariaceae	L, R	For sprains, swellings and sores (4)
Meriandera benghalensis (Roxb.) Benth	Mo-S01	Labiatae	L, F	Antiseptic for wounds and for urinary tract infections (4)
Nepeta deflersiana Schweinf. ex Hedge	Mo-S12	Labiatae	L	Antiseptic for wounds, rheumatic disorders, fever and colic (1, 3, 4)
Pollichia campestris Ait	Mo-A01	Caryophyllaceae	L, F	For sore throat and skin diseases (4)
Pulicaria inuloides DC.	Mo-M05	Compositae	L	For wounds (4)
Rhus retinorrhaea Steud. ex Oliv.	Mo-T22	Anacardiaceae	L	General tonic and for painful joints (4)
Solanum glabratum Dunal	Mo-M09	Solanaceae	L, T	Diuretic, for scabies, syphilis, cough and hemorrhoids (1, 2, 3, 4)
Tarconanthus camphoratus L.	Mo-S15	Compositae	L, F	For wounds and for urinary tract infections (4)
Teucrium yemense Deflers	Mo-S17	Labiatae	L, F	For kidney diseases and rheumatism (4)
Vernonia leopoldii Vatke	Mo-T16	Compositae	L, F	For cough, colic and skin diseases (3, 4)

B: Bark, F: Flower, L: Leaves, R: Roots or rhizomes, S: Stems, T: Fruits

[a] Most of the information of traditional use has been taken from (1) Fleurentin and Pelt 1982, (2) Al-Dubai and Al-khulaidi 1996; (3) Schopen 1983 and (4) native people

Table 2: In vitro antiproliferative activities (IC_{50} values in μg/ml ± S.E.M.) of the crude methanolic extracts tested against three human cancer cell lines and results of phytochemical screening

Plant species	Extracts	Cell lines			Phytochemical screening
		5637 (μg/ml)	MCF-7 (μg/ml)	A-427 (μg/ml)	
Acalypha ciliata	Methanolic	>50	>50	>50	Tannins, terpenoids, flavonoids
Ajuga bracteosa	Methanolic	>50	>50	>50	Essential oil, terpenoids, flavonoids, iridoids
Barleria trispinosa	Methanolic	>50	>50	>50	Iridoids
Blepharis ciliaris	Methanolic	>50	>50	>50	Flavonoids, iridoids
Buddleja polystachya	Methanolic	>50	>50	>50	Saponins, iridoids
Centaurothamus maximus	Methanolic	9.4 ± 0.50	9.9 ± 3.64	11.5 ± 4.49	Sesquiterpene lactones
Chenopodium ambrosioides	Methanolic	>50	>50	>50	Essential oil, terpenoids
Coccinia grandis (Fruits)	Methanolic	>50	>50	>50	Phytosterols, terpenoids, flavonoids
Coccinia grandis (Leaves)	Methanolic	>50	>50	>50	Terpenoids, flavonoids
Costus arabicus	Methanolic	19.2 ± 7.39	17.7 ± 3.96	18.3 ± 3.55	Steroids, alkaloids
Cupressus sempervirens	Methanolic	16.2 ± 2.86	13.7 ± 3.28	10.1 ± 3.79	Essential oil, flavonoids, phenolic acids, tannins
Dichrocephala integrifolia	Methanolic	13.9 ± 2.17	20.8 ± 6.61	10.4 ± 4.45	Essential oil, flavonoids, sesquiterpene lactones
Dorstenia barnimiana	Methanolic	>50	>50	>50	Coumarins
Enicostemma verticillare	Methanolic	>50	>50	>50	Flavonoids, tannins
Euphorbia schimperi	Methanolic	23.2 ± 35.3	>50	1.9 ± 0.79	Terpenoids, flavonoids
Gomphocarpus fruticosus	Methanolic	5.5 ± 0.68	6.2 ± 1.60	1.2 ± 1.06	Cardenolides, terpenoids
Grewia erythraea	Methanolic	>50	>50	>50	Phytosterols, flavonoids, triterpenens
Kanahia laniflora	Methanolic	1.2 ± 0.24	1.3 ± 0.07	0.5 ± 0. 17	Cardenolides, terpenoids, flavonoids
Kniphofia sumarae	Methanolic	>50	>50	>50	Anthraquinons
Lavandula dentata	Methanolic	>50	>50	>50	Terpenoids, essential oil, flavonoids
Leucas inflata	Methanolic	>50	>50	>50	Flavonoids, essential oil, terpenoids
Lindenbergia indica	Methanolic	>50	>50	>50	Sterols, saponins, flavonoids
Meriandera benghalensis (Leaves)	Methanolic	>50	>50	>50	Essential oil, flavonoids, terpenoids
Meriandera benghalensis (Roots)	Methanolic	0.9 ± 0.42	0.4 ± 0.35	2.5 ± 1.12	Terpenoids, phytosterols
Nepeta deflersiana	Methanolic	>50	>50	>50	Essential oil, phytosterols, terpenoids
Pollichia campestris	Methanolic	>50	>50	>50	Phytosterols, flavonoids
Pulicaria inuloides	Methanolic	42.3 ± 7.40	48.9 ± 9.11	41.8 ± 6.88	Sesquiterpene lactones, flavonoids
Rhus retinorrhaea	Methanolic	>50	>50	>50	Flavonoids, terpenoids
Solanum glabratum	Methanolic	9.4 ± 1.58	8.6 ± 0.33	9.0 ± 0.92	Alkaloids, steroids
Tarconanthus camphorates	Methanolic	4.7 ± 0.63	5.1 ± 2.87	5.9 ± 1.79	Essential oil, sesquiterpene lactones, flavonoids
Teucrium yemense	Methanolic	>50	>50	>50	Essential oil, terpenoids
Vernonia leopoldii	Methanolic	34.2 ± 5.19	41.5 ± 6.85	39.6 ± 1.62	Terpenoids, phytosterols, flavonoids
Etoposide (μM)		0.54 ± 0.30	0.50 ± 0.19	0.13 ± 0.10	

IC_{50} values are averages from three independent experiments

and *Tarconanthus camphoratus* with IC_{50} values between 0.4 and 6.2 μg/ml (Table 2). Furthermore, the extracts of *Centaurothamus maximus*, *Costus arabicus*, *Cupressus sempervirens*, *Dichrocephala integrifolia*, *Euphorbia schimperi*, *Pulicaria inuloides*, *Solanum glabratum* and *Vernonia leopoldii* showed a pronounced cytotoxic effect against all cancer cell lines with IC_{50} values between 8.6 and 48.9 μg/ml (Table 2). Table 3 presents the results of antimicrobial activities of the crude extracts in agar diffusion method. The MIC values are listed in Table 4. In most cases, among the investigated extracts the methanolic extracts resulted in the highest antibacterial effect. Generally, antimicrobial activity of the studied plant extracts was exhibited mainly against the Gram-positive bacteria. Only one plant demonstrated antimicrobial effect against Gram-negative bacteria and *Candida* strain (Table 3). Moreover, it was astonishing to observe that the multiresistant *Staphylococcus* strains manifested more sensitivity to the investigated extracts than the other antibiotic susceptible Gram-positive bacteria. The greatest antimicrobial effect was observed for the methanolic extracts of *Centaurothamus maximus*, *Cupressus sempervirens*, *Meriandera benghalensis* (roots) and *Tarconanthus camphorates* with MIC values between 31.2 and 250 μg/ml (Table 4).

Furthermore, a remarkable antibacterial effect with inhibition zones wider than 15 mm and MIC values between 250 and 1000 μg/ml was found for the methanolic extracts of *Enicostemma verticillare*, *Nepeta deflersiana*, *Pulicaria inuloides*, *Teucrium yemense* and *Vernonia leopoldii*. The majority of the water extracts of the investigated plants did not express any antibacterial effect or exhibited only a weak one. In addition, it was interesting to note that only the methanolic and water extracts of *Cupressus sempervirens* showed antifungal activity against *Candida maltosa*.

The same trend was noted with the antioxidant activity, i.e. only the methanolic extracts showed a high a radical

Table 3: Antimicrobial activities of the crude extracts against different strains of bacteria and fungi. Inhibition zones are in mm

Plant species	Extracts	Extract yield in %	Microbial strains tested						Multiresistant strains tested		
			S.a.	*B.c.*	*M.f.*	*E.c.*	*P.ae.*	*C.m.*	*S.e.* 847	*S.h.* 535	*S.a.* NGR
Acalypha ciliata	Methanolic	10.86	11	8	14	–	–	–	14	–	20
	Hot aqueous	6.81	–	–	–	–	–	–	14	–	18
Ajuga bracteosa	Methanolic	10.94	12	–	12	–	–	–	–	–	20
	Hot aqueous	8.05	20	–	14	–	–	–	14	–	24
Barleria trispinosa	Methanolic	12.89	9	7	9	–	–	–	18	12	16
	Hot aqueous	9.16	–	–	–	–	–	–	10	–	14
Blepharis ciliaris	Methanolic	8.40	–	–	–	–	–	–	10	–	12
	Hot aqueous	3.92	–	–	–	–	–	–	–	–	–
Buddleja polystachya	Methanolic	8.90	11	7	12	–	–	–	11	–	12
	Hot aqueous	13.23	–	–	–	–	–	–	–	–	–
Centaurothamus maximus	Methanolic	16.81	20	17	23	–	–	–	21	16	26
	Hot aqueous	7.85	–	–	–	–	–	–	11	–	12
Chenopodium ambrosioides	Methanolic	9.02	–	–	–	–	–	–	–	–	–
	Hot aqueous	7.60	–	–	–	–	–	–	–	–	–
Coccinia grandis (Fruits)	Methanolic	26.32	13	10	18	–	–	–	12	10	16
	Hot aqueous	21.97	–	–		–	–	–	–	–	–
Coccinia grandis (Leaves)	Methanolic	12.15	10	8	16	–	–	–	10	–	14
	Hot aqueous	6.22	–	–	–	–	–	–	–	–	–
Costus arabicus	Methanolic	23.56	10	8	12	–	–	–	12	8	18
	Hot aqueous	54.02	–	–	–	–	–	–	–	–	–
Cupressus sempervirens	Methanolic	61.12	20	18	27	16	19	12	26	20	28
	Hot aqueous	17.48	16	12	20	10	12	9	30	20	30
Dichrocephala integrifolia	Methanolic	18.20	13	10	15	–	–	–	18	12	16
	Hot aqueous	9.10	–	–	–	–	–	–	16	–	20
Dorstenia barnimiana	Methanolic	7.52	–	–	–	–	–	–	–	–	–
	Hot aqueous	6.85	–	–	–	–	–	–	–	–	–
Enicostemma verticillare	Methanolic	34.50	17	13	15	–	–	–	10	–	16
	Hot aqueous	7.53	–	–	–	–	–	–	–	–	–
Euphorbia schimperi	Methanolic	21.16	12	–	12	–	–	–	12	–	15
	Hot aqueous	9.71	–	–	–	–	–	–	–	–	–
Gomphocarpus fruticosus	Methanolic	19.77	8	7	8	–	–	–	–	–	14
	Hot aqueous	10.70	–	–	–	–	–	–	–	–	–
Grewia erythraea	Methanolic	10.23	–	–	–	–	–	–	–	–	12
	Hot aqueous	4.50	–	–	–	–	–	–	–	–	–
Kanahia laniflora	Methanolic	21.16	12	8	14	–	–	–	14	–	14
	Hot aqueous	16.80	–	–	–	–	–	–	13	–	16
Kniphofia sumarae	Methanolic	15.74	11	7	14	–	–	–	12	–	14
	Hot aqueous	20.81	–	–	–	–	–	–	–	–	–
Lavandula dentata	Methanolic	26.86	13	9	16	–	–	–	16	10	20
	Hot aqueous	13.23	–	–	–	–	–	–	26	10	24
Leucas inflata	Methanolic	9.75	9	–	–	–	–	–	8	8	14
	Hot aqueous	3.53	12	–	15	–	–	–	–	–	12

Table 3: (continued)

Plant species	Extracts	Extract yield in %	Microbial strains tested						Multiresistant strains tested		
			S.a.	*B.c.*	*M.f.*	*E.c.*	*P.ae.*	*C.m.*	*S.e.* 847	*S.h.* 535	*S.a.* NGR
Lindenbergia indica	Methanolic	23.65	9	–	14	–	–	–	–	–	–
	Hot aqueous	10.50	–	–	–	–	–	–	–	–	–
Meriandera benghalensis	Methanolic	16.02	12	10	20	–	–	–	16	12	18
(Leaves)	Hot aqueous	9.84	–	–	–	–	–	–	8	–	10
Meriandera benghalensis	Methanolic	12.32	22	20	28	–	–	–	29	22	34
(Roots)	Hot aqueous	5.60				–	–	–	12	–	14
Nepeta deflersiana	Methanolic	9.55	15	12	17	9	11	–	12	10	18
	Hot aqueous	6.50	–	–	–	–	–	–	–	–	–
Pollichia campestris	Methanolic	14.28	–	–	–	–	–	–	–	–	–
	Hot aqueous	8.62	–	–	–	–	–	–	–	–	–
Pulicaria inuloides	Methanolic	13.51	16	13	20	–	–	–	20	16	24
	Hot aqueous	7.06	–	–	–	–	–	–	10	–	13
Rhus retinorrhaea	Methanolic	18.10	12	10	14	–	–	–	12	10	14
	Hot aqueous	5.11	12	9	13	–	–	–	24	15	26
Solanum glabratum	Methanolic	18.52	11	8	18	–	–	–	12	–	18
	Hot aqueous	8.18	–	–	–	–	–	–	14	–	16
Tarconanthus camphorates	Methanolic	13.26	22	20	28	–	–	–	20	17	25
	Hot aqueous	5.22	10	8	12	–	–	–	12	8	15
Teucrium yemense	Methanolic	13.51	21	14	21	9	11	–	18	18	26
	Hot aqueous	2.41	15	–	–	–	–	–	10	–	16
Vernonia leopoldii	Methanolic	20.35	21	15	21	–	–	–	18	14	20
	Hot aqueous	9.75	–	–	–	–	–	–	–	–	12
Ampicillin 10 μg/disc			28	26	30	N.T.	N.T.	N.T.	–	–	–
Gentamicin 10 μg/disc			N.T.	N.T.	N.T.	15	17	N.T.	N.T.	N.T.	N.T.
Amphotericin 10 μg/disc			N.T.	N.T.	N.T.	N.T.	N.T.	10	N.T.	N.T.	N.T.

Inhibition zones including the diameter of the paper disc (6 mm). Paper disces were impregnated with 4 mg of dried extract. *S.a.*: *Staphylococcus aureus* ATCC 6538; *B.c.*: *Bacillus subtilis* ATCC 6059; *M.f.*: *Micrococcus flavus* SBUG 16; *E.c.*: *Escherichia coli* ATCC 11229; *P.ae.*: *Pseudomonas aeruginosa* ATCC 27853; *C.m.*: *Candida maltosa* SBUG; *S.e.* 847: multiresistant *Staphylococcus epidermidis*; *S.h.* 535: multiresistant *Staphylococcus haemolyticus*; *S.a.* NGR: North German reference strain, multiresistant *Staphylococcus aureus*; –: no activity; N.T.: not tested

Table 4: Results of the free radical scavenging activity and MIC of the investigated crude extracts

Plant species	Extracts	Radical scavenging activity in %					MIC in µg/ml		
		10 (µg/ml)	50 (µg/ml)	100 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	*S. aureus*	*B. subtilis*	*M. flavus*
Acalypha ciliata	Methanolic	0.5	1.9	10.2	42.5	85.0	>1000	>1000	>1000
	Hot aqueous	1.0	0.8	1.2	5.6	9.5	>1000	>1000	>1000
Ajuga bracteosa	Methanolic	2.8	4.4	6.9	78.3	90.0	1000	>1000	500
	Hot aqueous	0.9	2.5	15.6	36.8	34.4	1000	1000	500
Barleria trispinosa	Methanolic	20.1	24.7	39.2	93.9	94.6	>1000	>1000	>1000
	Hot aqueous	2.5	6.4	15.2	20.8	36.8	>1000	>1000	>1000
Blepharis ciliaris	Methanolic	8.6	11.8	17.2	93.4	94.7	>1000	>1000	>1000
	Hot aqueous	1.5	3.9	4.6	30.8	37.1	>1000	>1000	>1000
Buddleja polystachya	Methanolic	11.9	18.7	34.1	90.1	95.0	>1000	>1000	>1000
	Hot aqueous	2.5	6.2	15.7	22.3	26.7	>1000	>1000	>1000
Centaurothamus maximus	Methanolic	13.0	34.5	48.3	88.1	91.5	125	250	125
	Hot aqueous	1.8	0.8	1.4	4.9	13.7	1000	1000	500
Chenopodium ambrosioides	Methanolic	12.4	23.0	31.9	59.8	94.0	>1000	>1000	>1000
	Hot aqueous	1.1	14.1	22.5	24.5	35.6	>1000	>1000	>1000
Coccinia grandis	Methanolic	0.8	0.3	3.3	14.6	29.4	500	500	250
(Fruits)	Hot aqueous	1.0	0.4	1.2	5.8	7.2	>1000	>1000	>1000
Coccinia grandis	Methanolic	0.5	1.1	11.5	80.7	94.5	1000	>1000	1000
(Leaves)	Hot aqueous	1.5	0.7	2.4	18.2	25.9	>1000	>1000	>1000
Costus arabicus	Methanolic	0.1	1.1	3.9	29.3	62.1	>1000	>1000	>1000
	Hot aqueous	0.3	0.8	1.4	4.8	10.2	>1000	>1000	>1000
Cupressus sempervirens	Methanolic	81.7	95.6	95.8	95.9	96.1	62.5	125	62.5
	Hot aqueous	12.3	19.8	42.5	55.2	65.8	500	1000	500
Dichrocephala integrifolia	Methanolic	0.6	24.2	46.6	92.7	95.3	500	1000	500
	Hot aqueous	2.0	1.8	14.5	30.0	36.8	>1000	>1000	>1000
Dorstenia barnimiana	Methanolic	3.6	6.8	10.9	24.0	48.7	>1000	>1000	>1000
	Hot aqueous	0.6	1.6	1.1	6.8	10.5	>1000	>1000	>1000
Enicostemma verticillare	Methanolic	0.6	1.6	5.1	14.9	35.9	500	1000	500
	Hot aqueous	0.1	0.3	0.9	5.8	7.1	>1000	>1000	>1000
Euphorbia schimperi	Methanolic	12.2	213.	49.3	70.9	95.0	1000	>1000	1000
	Hot aqueous	0.6	0.5	2.1	14.8	22.4	>1000	>1000	>1000
Gomphocarpus fruticosus	Methanolic	6.2	20.6	39.2	95.0	94.3	>1000	>1000	>1000
	Hot aqueous	1.8	1.5	15.8	25.0	34.8	>1000	>1000	>1000
Grewia erythraea	Methanolic	8.5	9.3	37.3	79.7	94..9	>1000	>1000	>1000
	Hot aqueous	1.7	1.4	15.5	32.4	35.7	>1000	>1000	>1000
Kanahia laniflora	Methanolic	1.4	7.6	18.9	74.3	93.2	>1000	>1000	1000
	Hot aqueous	0.9	0.6	4.1	18.4	21.2	>1000	>1000	>1000
Kniphofia sumarae	Methanolic	2.0	6.8	16.5	66.1	91.0	1000	>1000	1000
	Hot aqueous	0.2	0.9	4.2	16.0	19.9	>1000	>1000	>1000
Lavandula dentata	Methanolic	9.8	16.0	33.6	95.0	91.4	1000	>1000	1000
	Hot aqueous	2.2	2.5	14.4	32.2	38.5	>1000	>1000	>1000
Leucas inflata	Methanolic	6.6	15.6	22.5	47.0	80.8	>1000	>1000	>1000
	Hot aqueous	2.1	1.5	5.8	16.5	20.1	1000	>1000	1000
Lindenbergia indica	Methanolic	9.8	21.6	38.4	91.0	91.0	>1000	>1000	>1000
	Hot aqueous	2.2	1.9	5.9	17.1	23.5	>1000	>1000	>1000
Meriandera benghalensis	Methanolic	6.5	49.2	73.7	95.2	93.7	1000	>1000	500
(Leaves)	Hot aqueous	1.6	1.5	7.5	23.8	28.7	>1000	>1000	>1000
Meriandera benghalensis	Methanolic	26.0	54.8	92.1	93.3	95.2	62.5	62.5	31.2
(Roots)	Hot aqueous	4.3	3.5	10.2	39.9	38.8	1000	>1000	1000
Nepeta deflersiana	Methanolic	0.5	4.0	15.8	52.4	90.7	500	1000	500
	Hot aqueous	1.4	0.9	2.4	18.8	28.5	>1000	>1000	>1000
Pollichia campestris	Methanolic	10.8	23.7	37.4	39.7	695	>1000	>1000	>1000
	Hot aqueous	2.3	4.8	10.7	14.1	21.2	>1000	>1000	>1000
Pulicaria inuloides	Methanolic	11.6	45.0	95.4	94.5	92.3	500	1000	250
	Hot aqueous	1.5	1.6	9.2	11.2	25.6	>1000	>1000	>1000
Rhus retinorrhaea	Methanolic	10.6	54.8	90.4	95.3	95.2	1000	>1000	1000
	Hot aqueous	2.6	5.1	32.0	41.5	48.6	1000	>1000	1000
Solanum glabratum	Methanolic	4.8	9.9	16.0	55.0	92.7	1000	>1000	500
	Hot aqueous	1.5	2.1	7.5	19.4	35.8	>1000	>1000	>1000
Tarconanthus camphorates	Methanolic	3.6	8.0	32.6	93.3	93.1	125	250	125
	Hot aqueous	2.4	14.7	35.7	42.3	66.4	>1000	>1000	>1000
Teucrium yemense	Methanolic	5.1	8.2	26.8	93.2	93.0	500	500	250
	Hot aqueous	0.8	1.6	6.2	22.8	41.0	>1000	>1000	>1000
Vernonia leopoldii	Methanolic	5.1	9.1	13.6	93.0	92.5	500	1000	250
	Hot aqueous	1.4	2.2	4.2	25.8	38.9	>1000	>1000	>1000
Ascorbic acid		36.9	90.2	95.8	96.6	96.3			

scavenging effect. Most of crude methanolic extracts of the investigated plants revealed this effect at the highest concentrations tested, however. Extraordinary radical scavenging effect at low concentration (100 μg/ml) was observed with *Cupressus sempervirens*, *Meriandera benghalensis*, *Pulicaria inuloides* and *Rhus retinorrhaea* (95.8%, 92.1%, 95.4% and 90.4% respectively) (Table 4). Additionally, the chemical composition of the methanolic extracts is shown in Table 2. It is worth to be mentioned that this work represents the first report on cytotoxic, antimicrobial and antioxidant effects of the most part of the investigated plants. Although few plants are partly investigated, existing knowledge is in many cases very limited.

The cytotoxic activity of *C. maximus* was in agreement with the effect reported by Muhammad et al. (2003). It was revealed that the isolated sesquiterpene lactones from this plant are responsible for its effect. These compounds are likely to be the cause of the antimicrobial effect found in our screen.

The estimated antimicrobial and antioxidant effects of the alcoholic extract of *C. sempervirens* are in accordance with literature data (Ibrahim et al. 2007; Toroglu 2007). The essential oil, the flavonoids and phenolic acids could be responsible for these activities. The measured cytotoxic effect has not been reported earlier (IC_{50} values between 10.1 and 16.2 μg/ml).

The roots of *M. benghalensis* showed more interesting activities than the aerial part in all assays. A previous study has already reported the isolation of four abietane diterpenoids from the roots of *M. benghalensis* (Torre et al. 1992); however no pharmacological effects were determined for the plant or its isolated compounds. The methanolic extract of the roots was the most potent cytotoxic and antimicrobial among the investigated extracts (IC_{50} values between 0.4 and 2.5 μg/ml and MIC values between 31.2 and 62.5 μg/ml) and it seems likely that the biological effects observed in our screens for this plant are related to the presence of these colored diterpenoids.

The potent cytotoxic effect against all tested cancer cell lines of both Asclepiadaceae plants namely *G. fruticosus* and *K. laniflora* is principally attributed to the cardenolides present in these plants (Fouche et al. 2006; Clarkson et al. 2005). Both plants exhibited only a weak or moderate antibacterial effect as well as antioxidant effect at high concentrations.

Other promising sources of new cytotoxic and antimicrobial agents represent the species *D. integrifolia*, *T. camphorates* and *V. leopoldii,* which demonstrated more or less noteworthy cytotoxic and antimicrobial effects in our screen. The chemical composition of the essential oil of *D. integrifolia* was already studied and five sesquiterpene lactones were isolated (Kuiate et al. 1999; Morikawa et al. 2006). Obviously, the sesquiterpene lactones present in this plant are very likely to be responsible for the cytotoxic effect observed in our screen. Recently, Matasyoh et al. (2007) reported on the chemical composition of the essential oil of *T. camphorates* and its antimicrobial effect. The reported antimicrobial effect is in accordance with the obtained result in our screen. Furthermore, our phytochemical screening revealed the presence of sesquiterpene lactones, which can be presumed the cause for the remarkable cytotoxic effect noticed in our screen. While a survey of the literature on the species *V. leopoldii* gave no result, many other species of the genus *Vernonia* have been investigated pharmacologically and phytochemically (Erasto et al. 2006; Huo et al. 2008). These literature data described the isolation of cytotoxic and antimicrobial sesquiterpene lactones. Our phytochemical screen revealed the presence of several sesquiterpene lactones in *V. leopoldii*, which probably caused the antimicrobial and cytotoxic effects observed here.

Consequently, these results support the view that some of the investigated plants like *C. maximus*, *C. sempervirens*, *D. integrifolia*, *M. benghalensis*, *T. camphorates* and *V. leopoldii* can be regarded as promising sources of potential new cytotoxic, antimicrobial and antioxidant agents. In some cases, few cytotoxic or antimicrobial constituents in the plants have been identified to date, and it can be assumed that the active compounds have not been completely exploited. Furthermore, the results show that scientific studies carried out on medicinal plants having traditional claims of effectiveness can yield fruitful results. Current work is aimed at the isolation and identification of the active substances. Finally, the results of the present study provide evidence that Yemeni flora continues to offer an important asset to the health care in community in Yemen and to represent exceptional possibilities for the discovery of new structures with anticancer and antimicrobial properties.

3. Experimental

3.1. Plant materials

The plants were collected from different governorates of Yemen in July 2005 and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Part of the identification of the investigated plants was done by Priv.-Doz. Dr. Peter Koenig, at the botanical garden, Ernst-Moritz-Arndt-University, Greifswald, Germany. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

3.2. Extraction of plant materials

The air-dried and powdered plant materials (10 g of each) were extracted with 400 ml methanol (CH_3OH) in a Soxhlet apparatus for 8 h. The residue was dried over the night and then extracted with 250 ml water (H_2O) by using a shaking water-bath at 70 °C for 2 h and repeated for three times. The obtained methanolic and water extracts were filtered and evaporated by using a rotary evaporator and freeze dryer. The dried extracts were stored at −20 °C until used.

3.3. In vitro antiproliferative assay on human cancer cell lines

For the estimation of the *in vitro* cytotoxic potency of the investigated extracts, an established microtiter plate assay was used (Bracht et al. 2006) with three human cancer cell lines: one lung cancer (A-427), one urinary bladder cancer (5637) and one breast cancer (MCF-7) line. The cell lines were obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, FRG). Cytotoxicity determinations are based on cellular staining with crystal violet and were performed as previously described in detail (Bracht et al. 2006). Briefly, a volume of 100 μl of a cell suspension was seeded into 96-well microliter plates at a density of 1000 cell/well. Twenty-four hours later, cells were treated with the plant extracts at five dilutions and exposed continuously to the extracts for the next 96 h. At the end of the exposure time, the medium was removed and the cells were fixed with a glutaraldehyde solution. The cells were then stained with crystal violet and the optical density (OD) was measured at $\lambda = 570$ nm with a plate reader. The percent growth values were calculated by the following equation:

$$\text{Growth } (\%) = OD_t - OD_{c,0}/OD_c - OD_{c,0} \times 100$$

Where OD_t is the mean absorbance of the treated cells, OD_c is the mean absorbance of the controls, $OD_{c,0}$ is the mean absorbance at the time the extract was added. The IC_{50} values were estimated by a linear least-squares regression of the growth values versus the logarithm of the extract concentration; only concentrations that yielded growth values between 10% and 90% were used in the calculation. Results were obtained from three independent experiments.

3.4. Determination of antimicrobial activity

3.4.1. Test microorganisms

The following microorganisms were used as test organisms in the screening: *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6059),

Micrococcus flavus (SBUG 16), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida maltosa* (SBUG 700). In addition, three multiresistant *Staphylococcus* strains namely, *Staphylococcus epidermidis* 847, *Staphylococcus haemolyticus* 535, and *Staphylococcus aureus* North German Epidemic Strain (supply from the Institute of Hygiene of Mecklenburg-Vorpommern, Greifswald, Germany) were also employed as test organisms.

3.4.2. Antimicrobial assay

The disc-diffusion assay described by Bauer et al. (1966) was used to determine the antimicrobial potential of the investigated extracts. Nutrient agar (OXOID LTD, Basingstoke, Hampshire, England) was prepared by dissolving of 27 g/l in water. The sterile nutrient agar was inoculated with microbial cells (200 µl of microbial cell suspension in 20 ml agar medium) and poured into sterile petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with 20 µl of the extract solution (equivalent to 4 mg of the dried extract). The paper discs were allowed to evaporate and after that placed on the surface of the inoculated agar plates. Plates were kept for 2 h in a refrigerator to enable prediffusion of the extracts into the agar. Then, the plates were incubated overnight (18 h) at 37 °C. In contrast, *M. flavus* was incubated at room temperature for 48 h and *C. maltosa* was incubated at 28 °C for 48 h. Ampicillin, gentamicin and amphotericin B were used as positive control. Negative controls were performed using paper discs loaded with 20 µl of organic solvents (methanol and 5% ethanol). At the end of the incubation period the antimicrobiral activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the disc). Extracts giving an inhibition zone of 15 mm or more were considered to have antibacterial activity.

3.4.3. Broth micro-dilution assay for minimum inhibitory concentrations (MIC)

The broth micro-dilution method described by Mann and Markham (1998) was used with modifications to determine the MIC of extracts against the three standard Gram-positive strains. With sterile round-bottom 96-well plates, duplicate two-fold serial dilutions of extract (100 µl/well) were prepared in the appropriate broth containing 5% (v/v) DMSO to produce a concentration range of 2000 to 15.6 µg of extract/ml. Two-fold dilutions of ampicillin were used as a positive control. A bacterial cell suspension (prepared in the appropriate broth) of 100 µl, corresponding to 1×10^6 CFU/ml, was added in all wells except those in column 10, 11 and 12, which served as saline, extract and media sterility controls, respectively. Controls for bacterial growth without plant extract were also included on each plate. The final concentration of bacteria in the assay was 5×10^5 CFU/ml. The final concentration of extracts was 1000 to 7.8 µg/ml. Plates were then incubated at 37 °C for 18 h overnight. After incubation, the MIC of each extract was determined as the lowest concentration at which no growth was observed in the duplicate wells. A p-iodonitro-tetrazolium violet solution (20 µl 0.04%, w/v) (Sigma, USA) were then added to the wells. The plates were incubated for a further 30 min, and estimated visually for any change in color from yellow to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained yellow corresponded to the MIC. Experiments were performed in duplicate.

3.5. Determination of antioxidant activity (scavenging activity of DPPH radical)

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple colour, typical for free DPPH radical decays, and the change in absorbency at $\lambda = 517$ nm is followed specrophotometrically. This test provides information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as described by Brand et al. (1995). The methanolic and aqueous extracts were redissolved in methanol and 5% ethanol, respectively, and various concentrations (10, 50, 100, 500 and 1000 µg/ml) of each extract were used. The assay mixture contained in a total volume of 1 ml, 500 µl of the extract, 125 µl prepared DPPH (1 mM in methanol) and 375 µl solvent (methanol or 5% ethanol). After 30 min incubation at 25 °C, the decrease in absorbance was measured at $\lambda = 517$ nm. The radical scavenging activity was calculated from the equation:

$$\% \text{ of radical scavenging activity} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}} \times 100$$

3.6. Phytochemical screening of the methanolic extracts

The screening of chemical constituents was carried out with the methanol extracts by using chemical methods and thin-layer chromatography (TLC) according to the methodology reported previously (Wagner and Bladt 1996).

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