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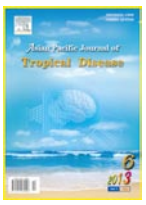

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Phytochemical screening, antimicrobial and antioxidant efficacy of different extracts of *Rumex dentatus* L. – A locally used medicinal herb of Kashmir Himalaya

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PEER REVIEW

Peer reviewer

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Comments

It is an outstanding piece of research and writing which evaluated the antimicrobial and antioxidant activity of different crude extracts of R. dentatus. The authors carried out an exhaustive, deep and wide-ranging lab work which clearly demonstrates the dose dependent relationship of the antimicrobial activity. The practical applicability of this work will benefit to mankind.
Details on Page 438

ABSTRACT

Objective: To elucidate the antimicrobial and antioxidant activities of *Rumex dentatus* L. (*R. dentatus*) along with its phytochemical analysis.
Methods: Agar disk diffusion method for antimicrobial activity and DPPH, riboflavin photo-oxidation, deoxyribose and lipid peroxidation assay for antioxidant activity.
Results: The antimicrobial and antioxidant activities of different concentrations of five *R. dentatus* extracts were tested against different clinical bacterial strains (*Shigella flexneri, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhimurium*) and fungal strains (*Aspergillus versicolor, Aspergillus flavus, Accremonium* spp., *Penicillium dimorphosporum, Candida albicans, Candida parapsilosis,* and *Candida kruesie*). Among all extracts, the butanol extract showed strong antibacterial activity against *Klebsiella pneumoniae* (inhibition zone diameter of 20 mm) and aqueous extract showed no activity against any of the bacterial strains. While as in case of the fungal strains, the maximum antifungal activity was observed against *Aspergillus flavus* by aqueous extract. The antioxidant activity revealed that the extracts exhibited scavenging effect in concentration-dependent manner on superoxide anion radicals and hydroxyl radicals. The phytochemical tests carried out with the crude extracts of *R. dentatus* showed the presence of flavonoids, terpenoids, alkaloids, saponins, tannins, anthraquinones and cardiac glycosides in it. The total phenolic content of these extracts was estimated quantitatively from standard calibration curve of gallic acid and it varied from 145 μg/mg in butanol extract to 45 μg/mg in petroleum ether extract.
Conclusions: It can be concluded that the plant has got a broad spectrum antimicrobial and antioxidant activity and could be used as a potential alternative for treating various diseases.

KEYWORDS

Antimicrobial activity, Antioxidant activity, *Rumex dentatus*, Medicinal herb

1. Introduction

Plants and plant derived products have been a source of medicine in the past centuries. Even today, scientists and the general public recognize their value as a source of new and complimentary medicines owing to their versatile applications[1]. Medicinal plants have been used for centuries as remedies for human diseases and offer a new source of biologically active chemical compounds as antimicrobial agents. Medicinal plants are the richest bio-resources of drugs for traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk

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medicines, pharmaceuticals and intermediate chemicals entitled for synthetic drugs[2,3]. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led scientists to investigate the antimicrobial activity of medicinal plants[4]. Likewise, the use of synthetic antioxidants are suspected to cause or promote negative health effects, hence stronger restrictions are being placed on their application and a trend to substitute them with naturally occurring antioxidants is developing[5]. The role of medicinal plants in disease prevention or control has been attributed to antioxidant properties of their constituents[6]. Keeping the above mentioned importance of medicinal plants in view, one of the medicinally important plants, *Rumex dentatus* L. (*R. dentatus*) used locally as a vegetable in Kashmir valleywas taken for the study. It belongs to the family Polygonaceae and is found throughout temperate western Himalayas, from Kashmir to Kumaon, 8 000-12 000 feet[7]. It contains a large number of chemically complex and biologically active compounds and is traditionally used as bactericidal[8], anti-inflammatory, anti-tumor, astringent , anti-dermatitis[9], diuretic, cholagogue, tonic and laxative agents[10].

2. Materials and methods

2.1. Plant material

R. dentatus L., a perennial or less commonly annual plant was collected as a whole plant locally from Srinagar and identified at Kashmir University Herbarium (KASH), Centre of Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar.

2.2. Extraction of plant material

The dried parts of the plant (50 g) were powdered and macerated. Crude extraction with solvents including petroleum ether, ethyl acetate, chloroform, butanol and aqueous was carried out in soxhlet extractor to get the respective extracts which were later dried, weighed and kept for further usage in sterilized caped vials at 4 °C.

2.3. Test organisms

The test microorganisms used in this study [bacteria: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella flexneri* (*S. flexneri*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Salmonella typhimurium* (*S. typhimurium*) and *Staphylococcus aureus* (*S. aureus*); fungi: *Aspergillus versicolor* (*A. versicolor*), *Aspergillus flavus* (*A. flavus*), *Penicillium dimorphosporum* (*P. dimorphosporum*), *Acremonium* spp., *Candida albicans* (*C. albicans*), *Candida kruesie* (*C. kruesie*) and *Candida parapsilosis* (*C. parapsilosis*)] were obtained from Bacteriological and Mycological section, Department of Microbiology, SKIMS, Soura, Srinagar.

2.4. Antimicrobial activity

The *in vitro* antibacterial activity test was carried out using the disk diffusion method[11].

2.5. Anti-oxidant activity assays

For evaluation of anti-oxidant activity of two alcoholic extracts, four methods were used.

2.5.1. DPPH assay

The anti-oxidant activity of both the extracts of the plant was measured with 1, 1-diphenyl 2-picryl hydrazyl radical (DPPH) spectrophotometrically at 517 nm[12]. The stock solution of both the plant extracts (5 mg/mL) was prepared by dissolving a known amount of dry extract in 10% aqueous dimethyl sulfoxide (DMSO). The working solutions (50, 100, 150, 200, 250 and 300 μg/mL) of all the extracts were prepared from the stock solution using suitable dilution. The scavenging activity was observed by bleaching of DPPH solution from violet colour to light yellow and ascorbic acid was used as control.

2.5.2. Superoxide anion radical scavenging activity

Measurement of superoxide anion scavenging activity of both the extracts of the plant was calculated spectrophotometrically at 590 nm using phosphate buffer (also taken as control) as blank after illumination for 5 min[13].

2.5.3. Hydroxyl scavenging activity

The colorimetric deoxyribose method was applied as the reference method of comparison for determining the hydroxyl radical scavenging activity of both the extracts of the plant at 532 nm[14].

2.5.4. Lipid peroxidation method

A modified thiobarbituric acid reactive species (TBARS) assay was used to measure the lipid peroxide formed using the egg yolk homogenate as lipid rich media at 532 nm[15].

The percentage of inhibition of the free radicals in the above mentioned methods was calculated by using the formula:

$$\text{Age inhibition } (\%) = \frac{(Ac - As)}{Ac} \times 100$$

where Ac was the absorbance of the blank and As was absorbance of sample.

2.6. Phytochemical analysis

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative

methods as described by various authors[2,16-19]. The plantextracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

3. Results

3.1. Antimicrobial activity

The antimicrobial activities of different concentrations (ranging from 150 μg/mL to 500 μg/mL) of various crude extracts of *R. dentatus* were determined against different bacterial and fungal strains and recorded as inhibition zone diameter (IZD), measured in "mm" with 10% aqueous DMSO as negative control, gentamycin as positive control for bacteria and nystatin for fungi (Tables 1 and 2). The butanol and ethyl acetate extracts of *R. dentatus* displayed promising antimicrobial activity against a wide range of bacteria and fungi, while as aqueous and petroleum ether extracts inhibited none of the tested bacterial strains. The inhibitory activity of all the extracts was found to be concentration-dependent. Petroleum ether extract showed no activity against any of the tested bacterial strains but showed some activity at the higher concentration (500 μg/mL) against *A. versicolor*, *Acremonium* spp. and *C. albicans* with a zone diameter of 14 mm, 16 mm and 9 mm respectively. The ethyl acetate extract showed inhibitory effect against some bacterial strains with highest inhibition zone diameter of 19 mm against *K. pneumonia* and *E. coli*. All fungal strains tested were found to be resistant against the extract at its lower concentration except for *C. albicans*, which was inhibited with a dameter zone of 11 mm at 250 μg/mL concentration. However, the higher concentration of this extract inhibited the growth of four fungal strains with a comparatively lower inhibitory effect.The chloroform extract was found effective against *K. pneumonia*, *S. typhimurium*, *S. aureus*, with IZD of 15 mm for *S. typhimurium*. It was completely ineffective against *S. flexneri*, *E. coli* and *P. aeruginosa*. When analysed against fungi, it was found effective only against *A. flavus* and *P. dimorphosporum*. Among all these extracts, butanol showed the highest antibacterial efficacy against *K. pneumoniae* with IZD of 20 mm, whereas the lowest efficacy against *S. aureus* (8 mm IZD) compared to the positive control (gentamycin 32-38 mm). *S. typhimurium* showed complete resistance against all concentrations of this extract. Of all the tested fungal strains, only *C. albicans* was inhibited by this extract with an IZD of 17 mm.

3.2. Antioxidant activity

The antioxidant activity of these extracts as measured by the ability to scavenge DPPH free radicals was compared with the standards ascorbic acid and butylated hydroxyl toluene (BHT). The results for the DPPH assay revealed that all the extracts exhibited significant antioxidant activity (Table 3). The highest percentage inhibition (92%) was shown by butanol extract compared to the positive control at 300 μg/mL followed by 86% by aqueous extract. Superoxide dismutase activity of different extracts determined by riboflavin photo-oxidation method depicted that the highest percentage inhibition (78%) by butanol extract at 300 μg/mL. However, the highest percentage inhibition (72%) for hydroxyl scavenging activity was exhibited by butanol extract at 300 μg/mL compared to the positive control (BHT: 95%) followed by 62% inhibition by aqueous extract, 49% inhibition by chloroform and 29%

Table 1
Antibacterial activity of extracts of *R. dentatus* (μg/mL).

Test organisms	Ethyl acetate			Chloroform			Butanol			Gentamycin
	150	250	500	150	250	500	150	250	500	
S. flexneri	10.00±0.57	12.00±0.57	14.0±1.0	–	–	–	11.0±1.0	18.00±1.73	18.00±0.57	37.0±1.0
K. pneumoniae	12.0±1.0	16.00±0.57	19.00±1.15	10.00±0.57	12.00±0.57	13.0±1.0	15.0±1.0	19.00±0.57	20.00±0.57	35.0±1.0
E. coli	–	18.0±1.0	19.00±1.73	–	–	–	15.00±0.57	17.00±0.57	18.0±1.0	30.00±1.15
P. aeruginosa	–	13.00±0.57	15.00±2.51	–	–	–	–	17.00±0.57	19.00±0.57	25.00±1.52
S. typhimurium	–	–	–	12.00±0.57	14.00±0.57	15.0±1.0	–	–	–	20.0±1.0
S. aureus	–	–	–	–	8.00±0.57	8.0±1.0	–	8.0±1.0	11.00±0.57	32.0±1.0

Table 2
Antifungal activity of extracts of *R. dentatus* (μg/mL).

Test organisms	Petroleum ether			Ethyl acetate			Chloroform			Butanol			Nystatin
	150	250	500	150	250	500	150	250	500	150	250	500	
A. versicolor	–	–	14.0±1.0	–	–	12.00±1.52	–	–	–	–	–	–	10.00±2.51
A. flavus	–	–	–	–	–	11.00±2.08	–	9.0±1.0	11.00±0.57	–	–	–	14.00±1.52
Acremoniumspp.	9.0±2.0	11.00±1.52	16.00±1.57	–	–	11.0±2.0	–	–	–	–	–	–	15.00±1.52
P. dimorphosporum	–	–	–	–	–	–	–	–	10.0±1.0	–	–	–	–
C. albicans	–	12.0±1.0	9.00±1.52	–	11.0±1.0	13.0±1.0	–	–	–	14.0±1.0	15.00±2.64	17.00±1.52	12.00±0.57
C. kruesie	–	–	–	–	–	–	–	–	–	–	–	–	21.00±1.73
C.parapsilosis	–	–	–	–	–	–	–	–	–	–	–	–	18.0±1.0

Table 3
Antioxidant activity (%) of extracts of *R. dentatus*.

Methods		Concentration (μg/mL)					
		50	100	150	200	250	300
DPPH assay	PE	–	–	–	23.00±1.52	27.00±0.57	30.00±0.57
	EA	–	–	–	–	25.00±1.52	29.00±2.08
	Chl	–	–	–	–	–	–
	B	65.00±1.15	71.00±0.57	78.00±0.57	83.0±1.0	25.00±1.52	92.00±0.57
	Aq	45.00±1.52	65.00±2.08	71.0±1.0	78.00±0.57	82.00±0.57	86.0±1.0
	AA	70.0±1.0	75.00±1.52	83.00±1.15	87.00±1.52	93.00±0.57	95.00±1.15
Riboflavin photo-oxidation method	PE	–	–	–	20.00±1.52	24.00±0.57	27.0±2.0
	EA	–	–	–	22.0±1.0	23.00±1.52	25.00±0.57
	Chl	–	–	–	–	–	–
	B	55.00±0.57	59.00±2.08	63.00±2.64	67.0±1.0	71.00±1.52	78.0±1.0
	Aq	44.00±1.52	50.00±1.52	53.0±1.0	59.00±1.52	62.00±0.57	66.0±1.0
	AA	65.0±1.0	71.00±1.52	80.00±1.52	87.0±1.0	94.00±0.57	97.0±1.0
Hydroxyl scavenging activity	PE	–	–	–	–	–	–
	EA	–	–	–	22.0±1.0	25.00±1.52	29.0±1.0
	Chl	–	–	33.00±1.52	37.00±0.57	43.00±0.57	49.0±1.0
	B	50.0±1.0	55.00±1.52	60.00±0.57	66.00±1.52	70.00±1.52	72.0±1.0
	Aq	40.00±1.52	44.0±1.0	49.00±1.15	53.0±1.0	57.0±1.0	62.00±0.57
	BHT	60.0±1.0	68.0±1.0	75.00±1.52	84.00±0.57	89.0±1.0	95.00±1.52
Lipid peroxidation method	PE	–	–	–	–	–	–
	EA	–	–	–	–	–	–
	Chl	–	–	–	–	–	–
	B	63.00±2.08	67.0±1.0	74.0±1.0	80.00±1.15	85.00±0.57	90.0±1.0
	Aq	49.00±1.52	53.00±1.73	59.0±1.0	62.00±2.51	66.00±2.08	70.0±2.0
	AA	59.00±1.52	64.00±2.08	70.0±1.0	75.0±2.0	81.0±1.0	89.00±2.08

PE: Petroleum ether, EA: Ethyl acetate, Chl: Chloroform, B: Butanol, Aq: Aqueous, AA: Ascorbic acid, BHT: Butylated hydroxy toluene.

inhibition by ethyl acetate extract.The effect of different plant extracts on *in vitro* inhibition of lipid peroxidation bybutanol extract was 90% followed by aqueous extract 70%. However, the ethyl acetate extract didn't exhibit any inhibition. The chloroform extract also displayed not any activity against the free radicals except for the hydroxyl ions for which it showed a mild inhibitory activity of 49% at 300 μg/mL in a concentration dependent manner compared to the positive control.

3.3. Qualitative analysis of phytochemical constituents

This phytochemical screening of different extracts of the plant showed that petroleum ether, chloroform and aqueous extracts tested were positive alkaloids. The extracts that tested positive for terpenoids included chloroform, butanol and aqueous. Flavonoids were found present in ethyl acetate, chloroform and butanol extracts. Likewise, the extracts positive for tannins were petroleum ether and chloroform (Table 4).

3.4. Quantitative estimation of phenolic compounds

The quantitative estimation of phenolic compounds measured as gallic acid equivalents depicted that the concentration of total phenolics was maximum for butanol (145 μg/mg), followed ethyl acetate extract (105 μg/mg), aqueous extracts (85 μg/mg) and chloroform extract (70 μg/mg). However, the lowest content of 45 μg/mg was found in the petroleum extract (Table 5).

Table 4
Qualitative analysis for various secondary metabolites in extracts of *R. dentatus*.

Phytochemicals	PE	EA	Chl	But	Aq
Alkaloids	+ve	−ve	+ve	−ve	+ve
Terpenoids	−ve	−ve	+ve	+ve	+ve
Flavonoids	−ve	+ve	+ve	+ve	−ve
Saponins	−ve	−ve	−ve	−ve	−ve
Tannins	+ve	−ve	+ve	−ve	−ve
Cardiac glycosides	−ve	−ve	−ve	−ve	−ve
Total Phenols	+ve	+ve	+ve	+ve	+ve

+ve: present; −ve: not present; PE: petroleum ether; EA: ethyl acetate; Chl: chloroform; But: butanol; Aq: aqueous.

Table 5
Total phenolic content of extracts of *R. dentatus*.

Extracts	Concentration (μg/mg GAEq)
Petroleum ether	45
Chloroform	70
Ethyl acetate	105
Butanol	145
Aqueous	85

GAEq: Gallic acid equivalent.

4. Discussion

Plants provide a large range of natural compounds belonging to different molecular families offering various medicinal properties. Ethno-botanical information revealed that the plant selected in this study is traditionally used for various medicinal purposes[20-23]. The antimicrobial activityof different plant extracts against the various clinical strains of bacteria and fungi supported the scientific validity of the plant being used traditionally as a medicine and vegetable. The results indicate that butanol yielded more potent extract with higher antimicrobial activity thus inhibiting the highest number of bacterial strains. This may also be attributed to the presence of soluble phenolic and polyphenolic compounds[24]. Rahmoun *et al*. and Vlachos *et al*. reported similar findings on the high antibacterial activity[25,26]. The results are also in confirmation with some recent studies[27,28]. The lack of antibacterial activity in some of the concentrations of the extract is not surprising as a number of plant extracts which have been found ineffectively against certain test organisms at lower concentrations and may be attributed to the presence of lesser amounts of the antimicrobial compounds. The antibacterial effects of the extracts could be explained by disturbance of the permeability barrier of the bacterial membrane structure[29]. All the extracts showed broad antimycotic activity against the tested fungal isolates. Most of the extracts of *R. dentatus*; namely petroleum ether, ethyl acetate and butanol inhibited clinical isolates of *C. albicans* that can be attributed to the presence of phenolic compounds. The amphipathicity of these compounds can explain their interactions with bio-membranes causing the inhibitory effect[30]. It was suggested that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, thus producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately their death[31]. It is evident from the results of the current study that susceptibility of pathogens to plant extracts depends upon solvent used for extraction, extract concentration and the organism tested as has been shown by many studies[32-34]. The aqueous extract of the plant inhibited none of the bacterial strains in comparison to the other extracts. This is in consonance with the results of a study reporting water to be less effective than organic solvents at extracting the active compounds from plants[35]. The results are also confirmed by a study showing the aqueous extract of *Jatropha curcus* as inactive against all the bacteria at all the concentration tested[36].

The free radical scavenging property of different extracts of *R. dentatus* evaluated using four different assays for each type of extract involves direct inhibition of the generation of reactive oxygen species, or the scavenging of free radicals. It is clear that a single method can not give a comprehensive prediction of antioxidant efficacy of the extracts. So, use of more than one method is recommended[37]. The DPPH free radical scavenging activity is due to the neutralization of DPPH free radical by the plant extract, either by transfer of hydrogen or of an electron[38]. The results show that butanol extract of *R. dentatus* may have hydrogen donors thus scavenging the free radical DPPH, with the highest scavenging activity than the other plant extracts, which may be attributed to the content of total phenolic compounds in them. The extracts of this plant scavenged free radicals in a dose-dependent manner corresponding with the results of various research works[39-41] showing that the plant metabolites such as flavonoids, tannins, catechins and other phenolic compounds possess antioxidant activity. The highest superoxide anion radical scavenging activity of butanol and aqueous extract of *R. dentatus* corroborates with the results of Jayasri *et al*[42]. The lipid peroxidation inhibitory activity of the plant extracts is a result of the effects of flavonoids on lipid peroxidation at the stage of initiation and termination of peroxyl radicals in confirmation with the findings of Paramaguru *et al*[43]. The extracts of this plant scavenged free radicals in a dose-dependent manner corresponding with many studies on *R. dentatus* and *Azadirachta indica*[44,45]. These findings are also supported by earlier reports that plant metabolites such as flavonoids, tannins, catechins and other phenolic compounds possess antioxidant activity[46].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

The plant kingdom still holds many species of plants containing substances of medicinal value that are yet to be

discovered. For this reason, *R. dentatus* L., a medicinal plant belonging to family Polygonaceae has been selected to be evaluated for antimicrobial and antioxidant activities. Preliminary phytochemical screening has also been carried out to detect the presence of phytochemicals that add to the medicinal value of the plant. The whole plant has been extracted with different solvents such as petroleum ether, ethyl acetate, chloroform, *etc*., for the determination of these activities.

Research frontiers

Cutting edge research in this paper is the evaluation of antimicrobial and antioxidant activity of different crude extracts of *R. dentatus*.

Related reports

A standard methodology has been followed to strongly test the hypothesis that *R. dentatus* is used as a medicinal herb in the folk medicine of Kashmir valley.

Innovations & breakthroughs

The nice part about the study is that it has been clearly demonstrated that the plant extracts contain some compounds which could be used as promising antimicrobial drugs and could also be used as strong antioxidant agents.

Applications

I feel that the research has got a good application in the drug manufacturing industry.

Peer review

It is an outstanding piece of research and writing which evaluated the antimicrobial and antioxidant activity of different crude extracts of *R. dentatus*. The authors carried out an exhaustive, deep and wide-ranging lab work which clearly demonstrates the dose dependent relationship of the antimicrobial activity. The practical applicability of this work will benefit to mankind.

References

[1] Liu ZL, He Q, Chu SS, Wang CF, Du SS, Deng ZW. Essential oil composition and larvicidal activity of *Saussurea lappa* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res* 2012; **110**(6): 2125-2130.

[2] Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *J Med Plant Res* 2010; **4**(2): 104-111.

[3] Anwer N, Waqar MA, Iqbal M, Mushtaq M, Sobia A. Phytochemical analysis, free radical scavenging capacity and antimicrobial properties of Impatiens bicolor plant. *Int Food Res J* 2013; **20**(1): 99-103.

[4] Rahmoun NM, Atmani ZB, Benabdallah M, Boucherit K, Villemin D, Braham NC. Antimicrobial activities of the henna extract and some synthetic naphthoquinones derivatives. *Am J Med Biol Res* 2013; **1**(1): 16-22.

[5] Aiyegoro OA, Okoh AI. Phytochemical screening and polyphenolic antioxidant activity of aqueous crude leaf extract of *Helichrysum pedunculatum*. *Int J Mol Sci* 2009; **10**(11): 4990-5001.

[6] Salazar-Aranda R, Pérez-López LA, López-Arroyo J, Alanís-Garza BA, Waksman de Torres N. Antimicrobial and antioxidant activities of plants from northeast of Mexico. *Evid Based Complement Alternat Med* 2011; doi: 10.1093/ecam/nep127.

[7] Kaul MK. Medicinal plants of kashmir and ladakh, temperate and cold-arid Himalaya. New Delhi: Indus Publishing Co.; 1997, p. 173.

[8] Mothana RAA, Abdo SAA, Hasson S, Althawab FMN, AlaghbariSAZ, Lindequist U. Antimicrobial,antioxidant and cytotoxic activities and phytochemicalscreening of some Yemeni medicinal plants. *Evid Based Complement Alternat Med* 2010; **7**(3): 323-330.

[9] Litvinenko YA, MuzychKina RA. Phytochemical investigation of biologically active substances in certain Kazakhstan *Rumex* species. *Chem Nat Comp* 2003; **39**(5): 368-370.

[10] Demirezer LO. Comparison of two *Rumex* species with spectrophotometric method and chromatographic identification with regard to anthraquinone derivatives. *Planta Med* 1993; **59**: 630.

[11] Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by standard single disc diffusion method. *Am J Clin Pathol* 1966; **36**: 493-496.

[12] Blois MS. Antioxidant determination by the use of a stable free radical. *Nature* 1958; **181**: 1199-1200.

[13] Liu SY, Sporer F, Wink M, Jourdane J, Henning R, Li YL, et al. Anthraquinones in *Rheum palmatum* and *Rumex dentatus* (Polygonaceae) and phorbol esters in *Jatropha curcas* (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails *Oncomelania*, *Biomphalaria* and *Bulinus*. *Trop Med Int Health* 1997; **2**(2): 179-188.

[14] Soobrattee MA, Bahorun T, Neergheen VS, Googoolye K, Aruoma OI. Assessment of the content of phenolics and antioxidant actions of the Rubiaceae, Ebenaceae, Celastraceae, Erythroxylaceae and Sterculaceae families of Mauritian endemic plants. *Toxicol In Vitro* 2008; **22**: 45-56.

[15] Padmaja M, Sravanthi M, Hemalatha KPJ. Evaluation of antioxidant activity of two Indian medicinal plants. *J Phytol* 2011; **3**(3): 86-91.

[16] Vogel AI. *A textbook of practical organic chemistry*. London: Longman; 1958, p. 90-92.

[17] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical

screening and extraction: A review. *Int Pharm Sci* 2011; **1**(1): 98-106.

[18] Rizk A, Bashir M. A chemical survey of sixty plants. *Fitoterapia* 1980; **53**: 35-44.

[19] Eleazu CO, Eleazu KC, Awa E, Chukwuma SC. Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. *J Biotechnol Pharm Res* 2012; **3**: 42-46.

[20] Hussain F. Allelopathic suppression of wheat and mustard by *Rumex dentatus* ssp. Klotzschianus. *J Plant Biol* 1997; **40**(2): 120-124.

[21] Mohan VR, Chenthurpandy P, Kalidass C. Pharmacognostic and phytochemical investigation of *Elephantopus scaber* L. (Asteraceae). *J Pharm Sci Technol* 2010; **2**(3): 191-197.

[22] Sher H, Hussain F. Ethnobotanical evaluation of some plant resources in Northern part of Pakistan. *Afr J Biotechnol* 2009; **8**(17): 4066-4076.

[23] Ali H, Sannai J, Sher H, Rashid A. Ethnobotanical profile of some plant resources in Malam Jabba valley of Swat, Pakistan. *J Med Plant Res* 2011; **5**(18): 4676-4687.

[24] Perez A, Orozco M, Rivas V, Waksman N. Experimental design to determine the factors affecting the preparation or extracts for antibacterial use. *Nat Prod Commun* 2008; **3**: 363-368.

[25] Rahmoun NM, Boucherit-Otmani Z, Boucherit K, Benabdallah M, Villemin D, Choukchou-Braham N. Antibacterial and antifungal activity of lawsone and novel naphthoquinone derivatives. *Med Mal Infect* 2012; **42**(6): 270-275.

[26] Vlachos V, Ciitchley AT, Von Holy A. Establishment of a protocol for testing antimicrobial activity in southern Africa macroalgae. *Microbios* 1996; **88**: 115-123.

[27] Bandh SA, Kamili AN, Ganai BA, Lone BA, Saleem S. Evaluation of antimicrobial activity of aqueous extracts of *Nepeta cataria*. *J Pharm Res* 2011; **4**(9): 3141-3142.

[28] Lone BA, Bandh SA, Chishti MZ, Bhat FA, Tak H, Nisa H. Anthelmintic and antimicrobial activity of methanolic and aqueous extracts of *Euphorbia helioscopia* L. *Trop Anim Health Pro* 2012; **45**: 743-749.

[29] Hayek SA, IbrahimSA. Antimicrobial activity of *Xoconostle* pears (Opuntiamatudae) against *Escherichia coli* O157:H7 in laboratory medium. *Int J Microbiol* 2012; doi: 10.1155/2012/368472.

[30] Moghadam MS, Maleki S, Darabpour E, Motamedi H, Mansour S, Nejad S. Antimicrobial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcus aureus* strains. *Asian Pac J Trop Med* 2010; **3**: 262-265.

[31] Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Antifungalactivity of *Ocimum sanctum* Linn. (Lamiaceae) on clinically isolated dermatophytic fungi. *Asian Pac J Trop Med* 2011; **4**(8): 654-657.

[32] Chaudhary G, Goyal S, Poonia P. *Lawsonia inermis* Linnaeus: A phytopharmacological review. *Int J Pharm Sci Drug Res* 2010; **2**(2): 91-98.

[33] Usman H, Abdulrahman FI, Usman A. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *Afr J Tradit Complement Altern Med* 2009; **6**(3): 289-295.

[34] Hadizadeh I, Pivastegan B, Hamzehzarghani H. Antifungal activity of essential oils from some medicinal plants of Iran against *Alternaria alternate*. *Am J Appl Sci* 2009; **6**: 857-861.

[35] Sukumarn S, Kiruba S, Mahesh M, Nisha SR, Miller PZ, Ben CP, et al. Phytochemical constituents and antibacterial efficacyof the flowers of *Peltophorum pterocarpum* (DC.) Baker ex Heyne. *Asian Pac J Trop Med* 2011; **4**(9): 735-738.

[36] Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). *Afr J Pharm Pharmacol* 2009; **3**(2): 58-62.

[37] Zadra M, Piana M, Brum TF, Boligon AA, Freitas RB, Machado MM, et al. Antioxidant activity and phytochemical composition of the leaves of *Solanum guaraniticum* A. St.-Hil. *Molecules* 2012; **17**: 12560-12574.

[38] Chairman K, Singh R, Alagumuthu G. Cytotoxic and antioxidant activity of selected marine sponges. *Asian Pac J Trop Dis* 2012; **2**(3): 234-238.

[39] Jhade D, Jain S, Jain A, Sharma P. Pharmacognostic screening, phytochemical evaluation and *in vitro* free radical scavenging activity of *Acacia leucophloea* root. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S501-S505.

[40] Mayakrishnan V, Veluswamy S, Sundaram KS, Kannappan P, Abdullah N. Free radical scavenging potential of *Lagenaria siceraria* (Molina) Standl fruits extract. *Asian Pac J Trop Med* 2012; **6**(1): 20-26.

[41] Kumari M, Jain S. Tannins: an antinutrient with positive effect to manage diabetes. *Res J Recent Sci* 2012; **1**(12): 70-73.

[42] Paramaguru R, Janaki S, Eswaran MB, Rao CV, Rawat AKS, Vijayakumar M. Free radical scavenging and lipid peroxidation inhibition potential of various fractions of *Murraya paniculata*. *Pharmacol* 2012; **3**(5): 138-143.

[43] Jayasri MA, Mathew L, Radha A. A report on antioxidant activity of leaves and rhizomes of *Costus pictus* D. Don. *Int J Integr Biol* 2009; **5**(1): 20-26.

[44] Humeera N, Kamili AN, Bandh SA, Shajr-ul-Amin, Lone BA, Gousia N. Antimicrobial and antioxidant activities of alcoholic extracts of *Rumex dentatus* L. *Microb Pathog* 2013; **57**: 17-20.

[45] Arokiyaraj S, Sripriya N, Bhagya R, Radhika B, Prameela L. Phytochemical screening, antibacterial and free radical scavengingeffects of *Artemisia nilagirica*, *Mimosa pudica* and *Clerodendrum siphonanthus* – An *in vitro* study. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 2): S601-S604.

[46] Yuan X, Gao M, Xiao H, Tan C, Du Y. Free radical scavenging activities and bioactive substances of Jerusalem artichoke (*Helianthus tuberosus* L.) leaves. *Food Chem* 2011; **133**: 10-14.