Antimicrobial Activity and Phyto-Chemical Analysis of an Alcoholic Extract of *Rumex dentatus* L.

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Abstract

Rumex dentatus- a medicinally important plant, belonging to family *Polygonaceae*, found throughout temperate western Himalayas, from Kashmir to Kumaon; contain a large number of chemically complex and biologically active compounds. The antimicrobial activity of various concentrations ranging from 150-500µg/ml of alcoholic (butanol) extract of *Rumex dentatus* L. was analyzed using Agar disk diffusion method on different clinical bacterial strains (*Shigella flexneri*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and fungal strains (*Aspergillus versicolor*, *A. flavus*, *Acremonium* spp., *Candida albicans*, and *C. kruesie*). The tested extract showed maximum antibacterial effect against *K. pneumoniae*. While as in case of the fungal strains the maximum antifungal activity was observed against *Candida albicans*. The phytochemical tests carried out on this crude extract showed the presence of Flavonoids, Terpenoids, Alkaloids, Saponins, Tannins, Anthraquinones and Cardiac glycosides. Total phenolic content of this extract; estimated quantitatively from standard calibration curve of Gallic acid showed a maximum yield of 145µg/mg. Thus, it can be concluded that the alcoholic extract has got a broad spectrum antimicrobial activity and could be a potential alternative for treating various diseases.

Keywords: Antimicrobial activity, phyto-chemical analysis, *Rumex dentatus*, extracts.

Introduction

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 of traditional medicinal systems, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals, and intermediate chemicals entitled for synthetic drugs (Hammer *et al*., 1999; Das *et al*., 2010). A whole range of plant derived dietary supplements, phytochemicals and pro-vitamins that assist in maintaining good health and combating diseases are now being described as functional ingredients and neutraceuticals. The potential of higher plants as source for new drugs is still largely unexplored and among the estimated 250,000 - 500,000 plant species, only a small percentage has been investigated phyto-chemically, with their fraction submitted to biological or pharmacological screening even smaller (Gerhartz *et al*., 1985; Kroschwitz *et al*., 1992). Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 1996). The WHO estimates that up to 80% of people still rely mainly on traditional remedies such as herbs for their medicine (Tripathi and Tripathi, 2003; Steenkamp *et al*., 2004). Therefore, attention to traditional medicine and the use of medicinal plants is being widespread and plants still represent a largest source of natural antioxidants and antimicrobial components (Sokmen *et al*., 1999; Conforti *et al*., 2008). Recently, 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 (Bisignano *et al*., 1996; Maoz and Neeman, 1998; Hammer *et al*., 1999; Das *et al*., 2010).

Nowadays, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious diseases. In addition to this, problems are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need to look for new and effective therapeutic agents which could be available from various medicinal plants (Khanahmadi *et al*., 2010). Reactive Oxygen Species (ROS) including free radicals such as (O_2- •, OH•) and non free radicals (H_2O_2, $^1O_2$) along with different forms of active oxygen are involved in diverse physico-chemical processes in the body (Qureshi *et al*., 2009) which have main role in the pathogenesis of different diseases, such as neurodegenerative disorders (Knight, 1997), diabetes, cancer (Dreher and Junod, 1996), cardiovascular diseases, atherosclerosis (Halliwell and Gutterridge, 1985), liver cirrhosis (Slater, 1987), cataracts and inflammation (Turkoglu *et al*., 2007; Conforti *et al*., 2008). The antioxidants prevent diseases by various mechanisms; by scavenging free radicals against oxidative stress and inhibiting lipid peroxidation (Miller and Rice-Evans, 1997) thus, their use in the form of herbal antioxidants in food and drug industries in the world is spreading widely (Kirca and Arslan, 2008). The protective effect of plant products in disease prevention are due to the presence of several components such as enzymes, proteins, vitamins (Halliwell, 1996), carotenoids (Edge *et al*., 1997), flavonoids (Zhang and Wang, 2002) and other phenolic compounds (Argolo *et al*., 2004). Plants offer a large range of natural compounds belonging to different molecular families, called Phytochemicals. These phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties, thus attracting several researchers to their elucidation to provide knowledge that will lead to advancement of medicine. There are more than thousand known phytochemicals produced from plants which help them to protect from various stresses and recent researches demonstrate, that they can protect humans against diseases as well.

Material and Methods

1.1 *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.

1.2 *Extraction of plant material*

The dried parts of the plant (50grams) were powdered and macerated. Crude extraction with butanol was carried out in a soxhlet extractor to get the respective extract which were later dried, weighed and kept for further usage in sterilized caped vials at 4 °C.

1.3 *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*) and *Staphylococcus aureus* (*S. aureus*); fungi: *Aspergillus versicolor* (*A. versicolor*), *Aspergillus flavus* (*A. flavus*), *Penicillium dimorphosporum* (*P. dimorphosporum*), *Acremonium* spp., *Candida albicans* (*C. albicans*) and *Candida kruesie* (*C. kruesie*) were obtained from Bacteriological and Mycological section, Department of Microbiology, SKIMS, Soura, Srinagar.

1.4 *Antimicrobial activity*

The *in vitro* antibacterial activity test was carried out using the disk diffusion method (Bauer *et al*., 1966).

1.5 *Phytochemical analysis*

Phytochemical analysis for major phytoconstituents of the plant extracts was undertaken using standard qualitative methods as described by various authors (Das *et al*., 2010; Vogel 1958; Rizk and Bashir, 1980; Tiwari *et al*., 2011;

Eleazu *et al*., 2012). The plant extracts were screened for the presence of biologically active compounds like glycosides, phenolics, alkaloids, tannins, flavonoids, saponins and steroids.

Results and Discussion

Several bench top assays such as antimicrobial assays including antibacterial and antifungal and phyto-chemical tests for alkaloids, terpenoids, flavonoids, saponins, tannins, anthraquinones, cardiac glycosides and total phenols of *R. dentatus* were carried out in the study. The Total phenolic content of all the extracts of the plant was determined according to Folin-Ciocalteu procedure (Padmaja *et al*., 2011). The observations were recorded and enlisted under following headings.

3.1 *Total yield of plant extracts*

The %age yield of crude extracts of *Rumex dentatus* as obtained by soxhlet extraction process and their morphological characteristics is depicted in Table 1. Under present study, butanol extract of whole plant showed a yield of 4.8G.

Table 1. Extraction yield and macroscopic characteristics of the crude extract of *R. dentatus*

Solvent	Plant part used	Colour	Odour	Consistency	Extracted yield (G)
Butanol	Whole plant	Dark green	Characteristic	Thick viscous fluid	4.8

3.2. *Qualitative analysis of phyto-chemical constituents*

The phytochemical tests were carried out with crude extract of *R. dentatus* to indicate the presence or absence of Flavonoids, Terpenoids, Alkaloids, Saponins, Tannins, Anthraquinones and Cardiac glycosides in it (Table 2). This phytochemical screening indicated that the extract tested positive for alkaloids, terpenoids, flavonoids, tannins and total phenols. The extract was negative for saponins, anthraquinones and cardiac glycosides.

Table 2. Qualitative analysis for various secondary metabolites in extract of *R. dentatus*

Phytochemicals		Butanol
Alkaloids	Dragendorff's Test	+ve
Terpenoids	Salkowski test	+ve
Flavonoids	Alkaline Reagent Test	+ve
Saponins	Froth Test	-ve
Tannins	Ferric Chloride Test	+ve
Anthraquinones		-ve
Cardiac glycosides	Keller-Killiani Test	-ve
Total Phenols	Ferric Chloride Test	+ve

'+ve' = presence; '-ve'= absence

3.3 *Quantitative estimation of phenolic compounds*

The total phenolic content of crude extract of *R. dentatus* (Table 3) was estimated quantitatively from standard calibration curve (Fig. 1). For butanol extract the total phenolic content was found to be 145µg/mg.

Table 3. Total phenolic content of extract of *R. dentatus*

Plant Extracts	Concentration (µg/mg GAEq)
Butanol	145

***GAEq – Gallic Acid Equivalent**

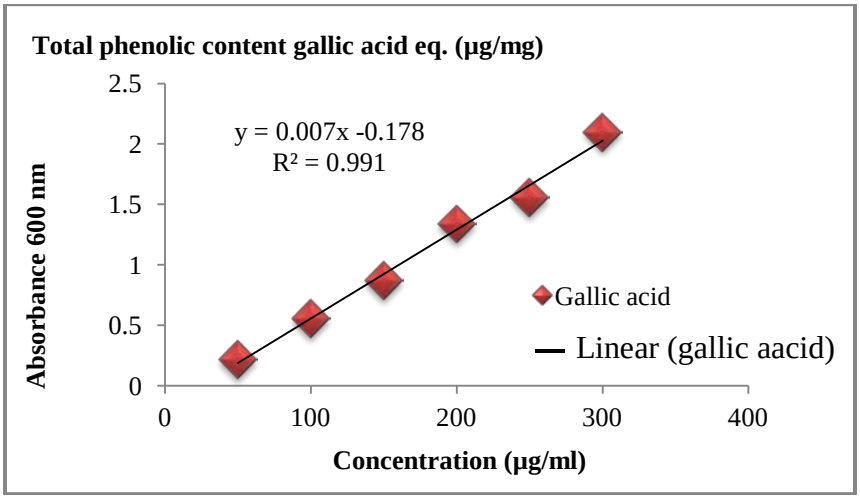

Fig. 1. Standard calibration curve for total phenolic content expressed as gallic acid equivalent

3.4 *Antimicrobial Activity*

The antimicrobial activities of different concentrations (ranging from 150 µg/mL to 500 µg/mL) of crude extract of *R. dentatus* viz., butanol was 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 4 and 5). The butanol extract of *R. dentatus* displayed promising antimicrobial activity against a wide range of bacteria. Of all the tested fungal strains, only *C. albicans* was found to be inhibited by butanol extract in comparison to the positive control nystatin.

Table 4. Antibacterial activity of alcoholic extract of *R. dentatus*

Test Organisms	Butanol			Gentamycin
	150µg/ml	250 µg/ml	500 µg/ml	
S. flexneri	11±1.0	18±1.73	18±0.57	37±1.0
K. pneumoniae	15±1.0	19±0.57	20±0.57	35±1.0
E. coli	15±0.57	17±0.57	18±1.0	30±1.15
P. aeruginosa	-	17±0.57	19±0.57	25±1.52
S. aureus	-	8±1.0	11±0.57	32±1.0

Table 5. Antifungal activity of alcoholic extract of *R. dentatus*

Test Organisms	Butanol			Nystatin
	150µg/ml	250µg/ml	500µg/ml	
A. versicolor	-	-	-	10±2.51
A. flavus	-	-	-	14±1.52
Acremonium sp.	-	-	-	15±1.52
C. albicans	14±1.0	15±2.64	17±1.52	12±0.57
C. kruesie	-	-	-	21±1.73

The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued in the management of plant and human disease management. Plants provide a large range of natural compounds belonging to different molecular families which offer various medicinal properties to humans. These molecules possess interesting biological activities which attracted several researchers to their elucidation to provide knowledge that will lead to advancement in medicine. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial and antioxidant efficacies will be used for the treatment of bacterial infections and other diseases in man (Balandrin *et al*., 1985). Ethno-botanical information revealed that the plant selected in this study is traditionally used for various medicinal purposes (Hussain *et al*., 1997; Liu *et al*., 1997; Yildirim *et al*., 2001; Manandhar, 2002; Hussain *et al*., 2006; Islam *et al*., 2006).

Phytochemical screening of different extracts of *R. dentatus* done as described in literature (Ayoola *et al*., 2008) revealed the presence of various biologically active compounds like flavonoids, terpenoids, saponins, alkaloids, tannins, cardiac glycosides, phenols and anthraquinones in the crude extract. The total phenols extracted and quantified according to Folin-Ciocalteu method (Padmaja *et al*., 2011), in terms of Gallic acid equivalents (GAE) showed highest concentration of phenols (145µg/mg of GAE) for butanol extract. It is well-known that phenolic

compounds contribute to quality and nutritional value in terms of modifying colour, taste, aroma, and flavor and also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to prevent molecular damage and damage by microorganisms, insects, and herbivores (Vaya *et al*., 1997). Secondary metabolites of plant origin appear to be one of the alternatives for the control of antibiotic resistant human pathogens. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Thus, antibacterial activity may be due to the presence of secondary metabolites (Arokiyaraj *et al*., 2009). The results of the phytochemical analysis in butanol extract as recorded in Table 2. Anthraquinones were absent in both this extract of *R. dentatus*, which is in accordance with the study conducted by Hariprasad and Ramakrishnan, (2011). They reported complete absence of alkaloids and anthraquinones in all the extracts of *Rumex vesicarius* L. The presence of these biologically active phytochemicals in extract of *R. dentatus* is also confirmed by the study conducted by Fatima *et al*., (2009) showing that alkaloids and saponins were only present in methanol extracts of leaves, shoots and roots of *R. dentatus*; anthraquinones and tannins were present only in alcoholic extract.

The butanol extract of *R. dentatus* displayed promising antimicrobial activity against a wide range of bacteria and fungi that were tested. Butanol extract inhibited maximum number of bacterial strains. The results indicate that butanol yielded more potent extract with higher antimicrobial activity thus inhibiting the highest number of bacterial strains, as in consonance with the study of Humeera *et al*., (2013). Rabe and Van Staden, (1997) and Vlachos *et al*., (1996) reported similar findings on the high antibacterial activity. This may also be attributed to the presence of soluble phenolic and polyphenolic compounds (Kowalski and Kedzia, 2007). The results are also in confirmation with a recent study of Bandh *et al*., (2011). George *et al*., (2002) explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. This extract of *R. dentatus*; namely butanol inhibited clinical isolate of *C. albicans* only. The inhibition zones produced by this extract for *C. albicans* was significantly higher as compared to the standard antibiotic used. Several studies have attributed the antifungal activity of plant extract to the presence of saponins (Aboaba and Efuwape, 2001; Mohanta *et al*., 2007). Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use against fungal pathogens of man (Cushnie and Lamb, 2005). Several studies have been conducted to understand the mechanism of action of plant extracts, but it is still unclear (Hadizadeh *et al*., 2009). However, some researchers attributed the antifungal activity to the phenolic compounds. The amphipathicity of these compounds can explain their interactions with bio-membranes causing the inhibitory effect (Veldhuizen *et al*., 2006). Omidbeygi *et al*., (2007) suggested that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, so producing a flux of protons towards the cell exterior which induces changes in the cells and, ultimately their death. Sharma and Tripathi, (2006) concluded that plant extracts may act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and integrity of the hyphal cell wall, resulting in its collapse and death of the mycelium. It is evident from the results of the current study that susceptibility of pathogens to plant extracts depends upon solvent used for extraction and extract concentration (Abou-Jawdah *et al*., 2002), as well as the organism tested (Kumaran *et al*., 2003; Hadizadeh *et al*., 2009). It is quite possible that the extract that was ineffective in some cases do not possess antibiotic properties, or they may have contained active constituents, just not in sufficient concentrations so as to be effective. It is also possible that some of the active chemical constituents were not soluble in this plant extract. The drying process may have caused conformational changes in some of the chemical constituents found in this plant. So, it is not surprising that there are differences in the antimicrobial effects of the extracts of the medicinal plants, due to the phytochemical properties (Stainer *et al*., 1986).

The antibacterial activity against a wide range of pathogens in this study makes *R. dentatus* a promising plant for further pharmacological investigations. The aim of this study was achieved by proving the *in-vitro* antimicrobial activity of alcoholic extract concentrations of this plant used traditionally as a folklore medicine. This study may contribute to the increased scientific investigation done on indigenous medicinal plants, including *R. dentatus* used traditionally in different parts of Kashmir valley.

Conclusions

It can be concluded that the alcoholic extract of plant has got a broad spectrum antimicrobial activity which is shown by the presence of different phytochemicals in this extract and thus, the plant could be used as a potential alternative for treating various diseases.

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