CONSORTIUM

History:
Received: June 4, 2015
Accepted: June 26, 2015
First Published: July 1, 2015
Collection year: 2015
Confirmation of publication: Published

Identifiers and Pagination:
Year: 2015
Volume / Issue: 7/3
First Page: 152
Last Page: 155
Publisher Id: JAppPharm-7-3
DOI:http://dx.doi.org/10.21065/19204159

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Citation:
Sulayman Abid, Saad Touqeer. Antimicrobial and antioxidant activity of breynia disticha and vernonia elaeagnifolia. J App Pharm (2015) 7:3. 152-155

Original Article

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *BREYNIA DISTICHA* AND *VERNONIA ELAEAGNIFOLIA*

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ABSTRACT:

The present study was aimed to investigate the phytochemistry and to determine the antibacterial, antifungal and antioxidant activities of the methanolic extract of the two plants namely, *Breynia disticha* and *Vernonia elaeagnifolia*. Antimicrobial activity was determined using disk diffusion method whereas antioxidant activity was carried out using DPPH assay. Antimicrobial activity was absent in *B. disticha* whereas *V. elaeagnifolia* possessed antibacterial activity against *P. mirabilis* (9.0±0.2 mm) and antifungal activity against *S. cerevisae* (8.0±0.1 mm). Both plants possessed significant antioxidant activities (more than 90% scavenging) comparable to the standard drug BHT. The study proves that both plants have high medicinal value.

***Keywords*:** Antibacterial; Antifungal; Plant; Extract; Activity; Phytochemistry; DPPH.

RUNNING TITLE: ANTIMICROBIAL AND ANTIOXIDANT STUDY

INTRODUCTION:

Microbial infections are one of the leading causes of death worldwide. The pathogenic microorganisms are becoming resistant to many of the commercially available antimicrobial drugs. Efforts by scientists in the area of chemotherapy have been increased to a great extent in the last two decades [1,2]. Natural products can be a good source of obtaining highly safe, potent and low cost antibacterial and antifungal drugs.

Reactive oxygen species (ROS) are the metabolic by-products produced in the human body. These free radicals are very harmful to our body and can damage the cell membrane and nucleic acid of our body if produced in excess amount. In order to reduce the oxidative stress caused by various endogenous and exogenous free radicals, antioxidant compounds are used. These compounds react with free radicals and neutralize them. Most of the potent antioxidants currently available are capable of scavenging a variety of ROS in both *in-vitro* and *in-vivo* conditions [3].

Breynia disticha is a shrub belonging to the family Phyllanthaceae. It is commonly found in gardens and public parks. The leaves of the plant are multicolored with green, purple and white to be most prominent of all the colors. *Vernonia elaeagnifolia* is creeper and belongs to family Asteraceae. It is commonly grown in gardens and lawns for screening purposes. The plant has been traditionally used as a leech repellant [4,5,6].

Until present, no study has been carried out in order to determine the antimicrobial and antioxidant activity of the plants. The aim of the present work is to report the antibacterial, antifungal, antioxidant and free radical scavenging effect of the plants for the first time.

MATERIALS & METHODS:

Plant material

The aerial parts of *Breynia disticha* and *Vernonia elaeagnifolia* were collected during the winter season from different parts of Lahore, Pakistan. The plants were identified by Dr. Ajaib Choudhary, Department of Botany, Government College University, Lahore. The voucher numbers received for *B. disticha* and *V. elaeagnifolia* were GC.Bot.Herb. 2282 and GC.Bot.Herb. 2283 respectively.

CONSORTIUM

Reviewing editor:
Taha Nazir, Ph.D. Scientific Executive ICDTD Inc. Saskatoon Saskatchewan Canada.: E. taha@icdtdi.ca

Funding:
The authors received no direct funding for this research.

Competing Interests:
The authors declare no competing interests

Additional information is available at the end of the article.

Preparation of extract

The plant material was shade dried and ground into coarse powder. It was extracted by cold maceration twice (7 days each) using methanol. The extracts obtained were dried using rotary evaporator and stored in air tight container in a refrigerator until further use.

Chemicals

Methanol of analytical grade was purchased from Panreac, Spain. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated HydroxyToluene (BHT) and Dimethly sulfoxide (DMSO) were purchased from Sigma-Aldrich, USA. All chemicals used in the phytochemical tests were of analytical grade.

Microbial strains

The clinical strains of *Klebsiella pneumonia* and *Proteus mirabilis* were used in the antibacterial assay whereas the clinical strains of *Aspergillus flavus* and *Saccharomyces cerevisae* were used in the antifungal assay. All the microbial strains were kindly supplied by Institute of Molecular Biology and Biotechnology, University of Lahore.

Phytochemical studies

Phytochemical tests for the identification of alkaloids, glycosides, tannins, flavonoids and saponins were performed according to the method described by Raaman [7].

Antibacterial activity

Antibacterial activity was determined by disk diffusion method described by Saad et al., [8]. Bacterial strains cultured in nutrient broth were spread over the surface of prepared Mueller Hilton Agar (MHA) media in sterilized Petri dishes. Sterile paper disks (6.0mm diameter) were then placed and 20µl of sample (20mg/1000 µl DMSO) was applied. The plates were incubated at 37ºC for 24h and the diameter of zone of inhibition was recorded.

Antifungal activity

Antifungal activity was also determined by disk diffusion method [8]. The fungal culture prepared in normal saline was spread over the surface of Sabouraud Dextrose Agar (SDA) media. To the paper disks, 20µl of sample (20mg/1000 µl DMSO) was loaded and then kept at room temperature for 24h. The diameter of zone of inhibition was determined.

Antioxidant activity

The antioxidant activity of methanolic extracts of plants was determined by spectrophotometric method [9]. The extracts used in the assay were dissolved in methanol to obtain a concentration of 1mg/ml. To 1ml of the sample solution 3 ml of Methanolic solution of DPPH (0.1mM) was added and incubated at 27°C for 20min in order to complete the reaction. The absorbance was determined at 517nm. BHT was used as a standard drug and the assay was carried out in triplicate.

Percentage scavenging was calculated using the following formula:

$$\% \text{ age scavenging} = [\text{Abs (control)} - \text{Abs (sample)} / \text{Abs (control)}] \times 100$$

RESULTS & DISCUSSION:

The results of preliminary phytochemical study are given in table 1. All major secondary metabolites were found in *Breynia disticha* methanolic extract (BDME) except for alkaloids. In case of *Vernonia elaeagnifolia* methanolic extract (VEME) glycosides and saponins were found to be absent. The presence of secondary metabolites indicates the importance of the two plants as therapeutic agents.

Table 1: Results of phytochemical studies.

Secondary Metabolite class	Test	BDME	
Alkaloids	Dragendorff's test	-	
Glycosides	Borntrager's test	+	
Phenolics and tannins	Ferric chloride test	+	
Flavonoids	Alkaline reagent test	+	
Saponin	Frothing test	+	

BDME, *Breynia disticha* methanolic extract; **VEME,** *Vernonia elaeagnefolia* methanolic extract; "+" indicates presence whereas "–" indicates absence.

The results of antimicrobial assay are given in table 2. BDME possessed no antibacterial or antifungal activity. In case of VEME, antibacterial activity was found against *P. mirabilis* with zone of inhibition of 9.0±0.2 mm and antifungal against *S. cerevisae* with zone of inhibition of 8.0±0.1 mm. The presence of antibacterial and antifungal activity in *Vernonia elaeagnefolia* indicates its usefulness as an effective antimicrobial agent against infectious clinical strains. The study can be further extended to standard strains to confirm the antimicrobial properties of the plant. The results of standard drugs Chloramphenicol (30µg) and Amphotericin (10µg) are also given in the table.

Table 2: Antibacterial and antifungal activity of methanolic extract of *B. disticha* and *V. elaeagnifolia*.

Microorganism	Microbial strain	Zone of inhibition (mm), Mean		
		BDME	VEME	
Bacteria	*K. pneumonia* (Clinical isolate)	-	-	
	P. mirabilis (Clinical isolate)	-	9.0±0.2	
Fungi	*A. flavus* (Clinical isolate)	-	-	
	S. cerevisae (Clinical isolate)	-	8.0±0.1	

BDME, *Breynia disticha* methanolic extract; **VEME,** *Vernonia elaeagnefolia* methanolic extract; a= Chloramphenicol (30µg); b= Amphotericin B (10 µg).

The results of antioxidant assay are given in table 3. The percentage scavenging of BDME was found to be 95.35±0.00 whereas that of VEME was calculated to be 93.80±1.35. The antioxidant activity of BDME was equal to the standard drug BHT while VEME possessed slightly lower antioxidant and free radical scavenging activity than the two samples. The results show the presence of highly significant antioxidant activity in the two plants.

Table 3: Antioxidant activity of methanolic extract of *B. disticha* and *V. elaeagnifolia*.

Sr. No.	Sample	%age Scavenging (Mean ± S.D)
1.	BDME	95.35±0.00
2.	VEME	93.80±1.35
3.	BHT	95.35±2.33

BDME, *Breynia disticha* methanolic extract; **VEME,** *Vernonia elaeagnefolia* methanolic extract; **BHT,** Butylated HydroxyToluene (standard)

DPPH assay is one of the easiest, quickest and most economical tests for the determination of antioxidant and free radical scavenging activity of plant extracts. Although this test alone is sufficient enough to establish the antioxidant value of plants however, some scientists perform additional tests to strengthen the results [10].

CONCLUSION:

Natural products are one of the best sources of antioxidants. These antioxidants not only prevent our body from harmful free radicals but also prevent different food products and pharmaceuticals from getting spoiled. The present study proves the presence of high antioxidant activity in the two plants and therefore their beneficial role as therapeutic agents for the first time. Further studies may be carried out for detailed extract characterization and isolation of compounds responsible for the biological activity.

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