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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Anabasis aphylla* L. EXTRACTS

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ABSTRACT. The present study was designed to evaluate the phytochemical screening, antimicrobial and antioxidant activities of *Anabasis aphylla* L. extracts. Phytochemical screening revealed the presence of alkaloid, flavonoid, saponin, terpenoid, steroid and sterols in the extracts of aerial parts of *A. aphylla*. The dry aerial part of *A. aphylla* was extracted with methanol, then further fractioned with n-hexan, ethyl acetate, n-butanol, and water. Four fractions (methanol, ethyl acetate, n- butanol, and water) were examined for antimicrobial activity by using disc diffusion assay. The microbial strains were exposed to the following four different concentrations of extracts: 1, 10, 25 and 100 mg/ml. There has been an increasing effect on microbial growth inhibition with increasing concentration of the extracts. The largest zone of inhibition was obtained with n-butanol extract against *Aspergillus niger* (20mm). The antimicrobial activity of extracts was compared with the standard antibiotics like amoxicillin and ketoconazol (100 µg/disc). Furtheremore, the antioxidant activity of two fractions (methanol and ethyl acetate) were tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid assays. The extracts showed no antioxidant activity in DPPH method. In β-carotene/linoleic acid test, the ethyl acetate (100 mg/ml) showed higher antioxidant activity (81.8%).

Key words: phytochemical, antimicrobial activity, antioxidant activity, *Anabasis aphylla.*

INTRODUCTION

The development of microbial resistance towards antibiotics has heightened the importance of the search for new potential effective plants and plant constituents against pathogenic microorganisms (AHMAD *et al*., 2006). The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and

protective/disease preventive properties (phytochemicals). These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (NONITA *et al*., 2010). Biologically active widespread from plant sources have always been of great interest to scientists working on infectious diseases. Over the past decade there has been an explosion of interest in the antimicrobial, particularly antibacterial and antifungal, activity of natural products (CLARK and HUFFORD, 1993). The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases (COOPER *et al*., 2006). Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery (CSEKE *et al*., 2006). Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (phytochemicals) (MONGOLE *et al*., 2010). The effect of plant extracts on microorganisms has been studied by a very large number of researches in different parts of the word (MAHESH and SATISH, 2008; OHIRI and UZODINMA, 2000; AMANLOU *et al*., 2004).

A. aphylla is an herbaceous perennial plant, belonging to the family Chenopodiaceae (MOZAFFARIAN, 2001). Several types of alkaloids from *A. aphylla* have been isolated and characterized (TILYABAEV and ABDUVAKHABOV, 1998). It is recognized that alkaloids have strong antimicrobial, antibacterial and antifungal biological properties (NANTACHIT *et al*., 2010; NETO *et al.*, 2011; DAN *et al*., 2008).

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may therefore have health-promoting effects in the prevention of degenerative diseases (SHAHIDi, 1997). There is a growing interest in natural antioxidants, present in medicinal and dietary plants that might help attenuate oxidative damage (SILVA *et al*., 2005).

The present study reports on the phytochemical analysis, antimicrobial and antioxidant activities of various extracts from *A. aphylla*.

MATERIALS AND METHODS

Collecting of plant materials

Aerial parts of *A. aphylla* were collected in the surroundings of Ghoochan (Iran) in August 2010. The plant material was identified and a voucher specimen has been deposited in the Herbarium of Sistan and Baluchestan University, Zahedan, Iran. The plant material was dried at room temperature and used for all the extracts prepared.

Preparation of extracts

Air-dried powder and ground plant material (500 g) were extracted by stirring with magnetic stirrer in methanol at room temperature for three days. Methanolic extract was filtered and concentrated using a rotary evaporator at 40 $^{\circ}$C. The resulting gummy mass was suspended in water and partitioned with n-hexan, ethyl acetate, n-butanol and water.

Phytochemical analysis

Preliminary screening of secondary metabolites such as alkaloids, flavonoids, saponins, coumarins, antraquinons, terpenoids, steroid and sterols were carried out according

to the common phytochemical methods described by TREASE and EVANS (1983) and HARBORNE (1973).

Antimicrobial activity

The four different concentrations of the extracts were tested for antimicrobial activity using disc diffusion assay according to the method of BAUER *et al*. (1966). The test microorganisms used in this study (bacteria: *Staphylococcus aureus* (PTCC 1764), *Enterococcus faecalis* (PTCC 1394), *Bacillus polymyxa* (ATCC 10401), *Pseudomonas aeruginosa* (CIP A22), *Salmonella typhi* (PTCC 1609), *Proteus mirabilis* (OXK PTCC 1076); fungi: *Aspergillus niger* (PTCC 5223), *Candida albicans* (PTCC 5027) were obtained from Iranian Research Organization for Science and Technology (IROST).

Disc diffusion assay

The strains of microorganism obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37 °C or 24 h and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter with had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 °C. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the discs using zone reader (mm). Amoxicillin and ketoconazol (100 µg/disc) were used as standards. Dimethylsulfoxide (DMSO) was used as negative control.

Antioxidant activity

Different concentration of both methanol and ethyl acetate extracts were prepared in methanol: 100, 50, 25, 12.5, 6.25, 3.12, 1.5, 0.75 and 0.3 mg/ml. The antioxidant activity of extracts was carried out using two different methods: free radical scavenging activity using DPPH and by the beta-carotene/linoleic acid assay.

DPPH assay

DPPH radical-scavenging activity was measured according to the method of SHIMADA *et al.* (1992) with slight modification. One ml of 0.1 mM DPPH solution in methanol was added to 1 ml of each sample at different concentrations, and then the mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance of the resultant solution was measured at 517 nm. The DPPH radical-scavenging activity was calculated as follows: scavenging activity (%) = $[(A_0 - (A - A_b)) / A_0] \times 100\%$, where A_0 was value of DPPH without sample; A was value of sample and DPPH; A_b was value of sample without DPPH. The synthetic antioxidant Butylated Hydroxy Toluene (BHT) was used as positive control.

Beta-carotene/linoleic acid assay

In this assay, antioxidant activity was determined by measuring the inhibition of conjugated diene hydroperoxides arising from linoleic acid oxidation (TEPE *et al.,* 2005). A stock solution of beta-carotene/linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 ml chloroform, and 25 µl of linoleic acid and 200 mg of Tween-40 were added as emulsifier since β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of oxygen-saturated distilled water was

added with vigorous shaking at a rate of 100 ml/min for 30 min; 2500 µl of this reaction mixture was dispensed into test tubes, and 350 µl volumes of extracts, prepared in various concentrations, were added. The emulsions were incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant activities of the extracts were compared with that of BHT and the blank. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

Thousands of diverse natural products are produced by plants and many of these are involved in plant defense. The phytochemical diversity of antimicrobial compounds include terpenoids, saponins, phenolics and phenyl propanoids, pterocarpans, stilbenes, alkaloids, glucosinolates, hydrogen cyanide, indole and also elemental sulphur, the sole inorganic compound (COOPER *et al*., 1996). In this study, the phytochemical analysis of the methanol extract of the *A. aphylla* (Table 1) showed the presence of different groups of secondary metabolites such as alkaloids, flavonoids, saponins, terpenoids, steroid and sterols which are of medicinal importance.

Table 1. - Phytochemical screening on the methanol extract of the aerial parts of *Anabasis aphylla*

Class of compounds	Methanol extract
Alkaloids	+*
Coumarins	–
Antraquinones	–
Saponins	+
Flavonoids	+
Terpenoids	+
Steroids and strols	+

* + = Presence of constituents; – = Absence of constituents.

The dried extracts were dissolved in DMSO to produce the required concentrations and were investigated for their antimicrobial activities by using test microorganisms. There has been an increasing effect on microbial growth inhibition with increasing concentration of the extracts. However, the effects observed were less than those produced by the standard agents. The results on antimicrobial activity of the *A. aphylla* extracts against eight microbial strains are presented in Table 2.

Our results showed that the n-butanol fraction was the most active, followed by the ethyl acetate, methanol and water fractions. Previous study on this plant showed the presence antibacterial phenolic compounds in ethyl acetate fraction (DU *et al.,* 2007).

The mean zone diameter inhibitions by n-butanol, ethyl acetate, methanol and water extracts were found to be 0-20, 0-19, 0-16 and 0-14 mm for both bacteria and fungi with increasing concentrations, respectively. The rational for this effect may be based on the ability of polar extract permeability through the plasma membrane. It seems hydrophobicity is not the sole determinant factor for the active stability of a membrane structure. Many other molecular mechanisms other than hydrophobicity are involved such as protein flexibility and solubility (MARJORIE, 1999).

Table 2. - Antimicrobial activity of *Anabasis aphylla* extracts.

Microorganism	Inhibition zone (mm)																		
	Methanol				Ethyl acetate				n-butanol				Water				Standards		Control
	1	10	25	100	1	10	25	100	1	10	25	100	1	10	25	100	Amoxicillin	Ketoconazol	DMSO
Staphylococcus aureus	0	0	0	8	0	0	0	10	0	0	7	10	0	0	0	0	20	-	-
Enterococcus faecalis	0	0	0	8	0	0	0	10	0	0	7	13	0	0	0	7	21	-	-
Bacillus polymyxa	0	0	0	9	0	0	7	11	0	0	8	15	0	0	0	9	24	-	-
Pseudomonas aeruginosa	0	0	7	11	0	0	7	14	0	0	7	10	0	0	0	7	22	-	-
Salmonella typhi	0	0	0	10	0	0	0	9	0	7	9	15	0	0	0	10	25	-	-
Proteus mirabilis	0	0	8	14	0	0	9	13	0	8	10	18	0	0	7	10	26	-	-
Aspergillus niger	0	7	8	15	0	7	0	18	0	7	12	20	0	0	8	14	-	30	
Candida albicans	0	7	8	17	0	7	10	19	0	8	10	18	0	0	7	12	-	28	

The n-butanol fraction at 100 mg/ml showed the maximum antibacterial activity against *Enterococcus faecalis* (13mm) and *Proteus mirabilis* (18 mm) and antifungal activity against *Aspergillus niger* (20 mm), while the ethyl acetate fraction at 100 mg/ml showed the maximum antibacterial activity against *Proteus mirabilis* (18 mm) and antifungal activity against *Candida albicans* (19 mm). At concentration of 1 mg/ml, all of these fractions did not show any antimicrobial activity. Water extracts exhibited antimicrobial activity at 25 and 100 mg/ml. As indicated in Table 2, water extracts at 0.1 mg/ml showed strong antifungal activity against *Aspergillus niger* (14 mm) and *Candida albicans* (12 mm) and antibacterial activity against *Salmonella typhi* (10 mm) and *Proteus mirabilis* (10 mm).

The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of this plant in the traditional uses for insecticide.

DMSO also had no effect on the growth of any of the eight microorganisms. Standard disc inhibited the growth of all the test microorganisms.

Literature has no report on the antioxidant activity of *A. aphylla* and this is the first one. Methanol and ethyl acetate extracts of *A. aphylla* were subjected to screening for their possible antioxidant activities using DPPH and β-caroten/linoleic acid assay methods at different concentrations. The extracts showed no antioxidant activity in DPPH method. The reason for this behavior is unclear for us. In the β-caroten/linoleic acid method, the antioxidant activity level of a substance is determined by measuring oxidation products of linoleic acid that simultaneously attack β-caroten, resulting in bleaching of its characteristic yellow color (GORINSTEIN *et al*., 2003; FUKUMOTO and MAZZA, 2000). Percent inhibition of linoleic acid oxidation of the extracts is shown in Figure 1. In both ethyl acetate and methanol extracts, there was an increasing in antioxidant activity with increasing concentration. For high concentrations (100 mg/ml) of ethyl acetate and methanol extracts, the antioxidant activity reached 81.8% and 79.3%, respectively. The antioxidant activity may be due to the presence of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups and other structure features (PATT *et al.,* 1990).

The best percentage of antioxidant activity was observed for BHT (93.6 %).

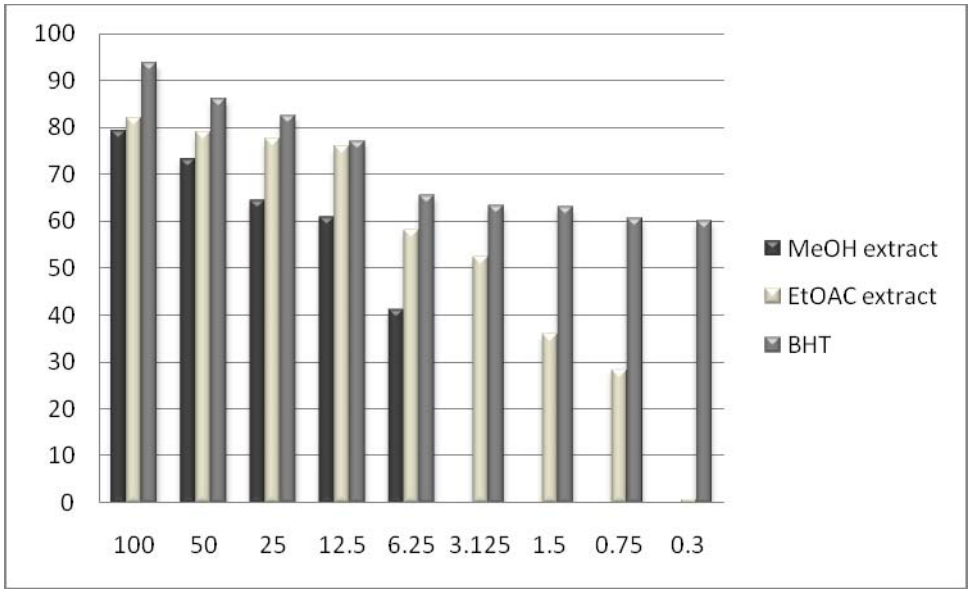

Figure 1. - Antioxidant activity of different concentrations of the extracts of *A. aphylla* and BHT measured by the β-carotene/linoleic acid method.

CONCLUSION

In this study, the antimicrobial and antioxidant activities of the extracts of *A. aphylla* were evaluated. Results for the antimicrobial activity were relatively close to the standards (amoxicillin and ketoconazole). In the antioxidant activity test (β-caroten/linoleic acid assay method), the highest inhibitory percent reached 81.8 % for methanol extract.

The demonstration of broad spectrum of antimicrobial activity by *Anabasis aphylla* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control.

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