Pak. J. Bot., 47(1): 121-124, 2015.

PHYTOCHEMICALS, ANTIBACTERIAL AND ANTIOXIDATIVE INVESTIGATIONS OF *ALHAGI MAURORUM* MEDIK.

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Abstract

Ethnomedicinally the plant *Alhagi maurorum* is used for diverse topical infections in the different culture of Khyber Pakhtunkhwa Pakistan. The aim of the present study is to look into the possible natural tharapy in the form of bioactive fractions which can be further subjected to the isolation of natural products leading towards drug discovery. The methanolic extract and its derived fractions {*n*-hexane, chloroform, ethyl acetate, *n*-butanol and residual aqueous fraction} of leaves, roots and flowers of *Alhagi maurorum* are subjected to microbicidy against *Salmonella typhe, Staphylococcus aureus, Vibrio cholerae, Shigella dysenteriae, E. coli* and *Bacillus anthrax,* antioxidant profile by DPPH method and preliminary phytochemical investigations. It is observed that the leaves of the plant showed outstanding response to most bacterial pathogens followed by roots while the fractions from flowers were almost inactive. The antibactrial profile of the plant leaves exhibited that the crude extract, chloroform and ethyl acetate fractions showed outsatandinding activities giving above 80% inhibition against *B. anthracis*. The crude extract showed 80% inhibition against *S. dysenteriae*. The ethyl acetate and crude extarct was also good against *S. typhe* with 78.35% and 76.50% inhibition respectively. Extracts/fractions from leaves of the plant showed strong radicle scaving activity, it may be due to the presences of phenolic compounds in plant. Phytochemical screening of crude extracts and its subsequent fractions demonstrated the presence of fats, alkaloids, flavonoids, anthraquinones, cardiac glycosides, coumarins, saponins, phlobatannins, tannins and terpenoids in leaves and roots while the flowers were found to be devoid of any such phytochemical.

Key words: *Alhagi maurorum*, Antibacterial activities, Antioxidant activities, Preliminary phytochemical evaluation, Drug findings.

Introduction

Since very old times, herbal medications have been used for relief of symptoms of disease (Shinwari *et al.,* 2006). Despite the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines as well as their prophylactic properties, especially in developing countries. Large number of medicinal plants has been investigated for their antibacterial and antioxidant properties. Natural medicinal agents either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by microbial pathogens and oxidative stress (Shinwari *et al.,* 2013). Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [Sarwat *et al*., 2012].

Alhagi maurorum commonly known as camelthorn is a perennial deciduous shrub and belongs to family Fabaceae. It grows up to 2 m. Its flowering season is July. The flowers are small, bright pink to maroon and hermaphrodite. The plant love to grow in sandy and loamy soils. It can fix nitrogen (Nasir & Ali, 1977). *Alhagi maurorum* has been used locally in folk medicine as a treatment for nasal polyps, glandular tumors and ailments related to the bile ducts. It is used as a medicinal herb for its diaphoretic, gastroprotective, diuretic, laxative, expectorant, antiseptic, antidiarrhoeal and healing of wounds. Oil from the leaves is used in the treatment of hemorrhoids and rheumatism. The flowers are used in the treatment of piles (James, 2011; Shinwari *et al.,* 2006).

Materials and Methods

Plant collection: The plant was collected in 2013 from ALGADI village of district Karak Khyber Pakhtunkhwa, Pakistan. The plant was botanically identified by the Curator, Department of Botany, Kohat University of Science and Technology with the help of available literature. A voucher specimen (accession #1233) was deposited at the herbarium of the department.

Extract preparation: The fresh plant parts leaves (4.5 kg), roots (5 kg) and flowers (2 kg) were collected and shade dried which were later coarsely powdered in a Willy Mill to 60-mesh size and used for solvent extraction. For sample preparation dried powdered samples were extracted thrice with methanol at room temperature for 21 days and concentrated using a rotary evaporator under reduced pressure to yield the crude extracts. The residue (crude extract) was suspended in water and partitioned successively with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and soluble residual aqueous fraction yielding respective fractions (Shinwari *et al.,* 2013).

Antibacterial assay: For antibacterial activities agar diffusion technique was used with little modifications as described by Shinwari *et al.* (2013). In this method, wells were prepared in petriplates, the required concentration of

stock solution were poured in these wells and after incubation of 24 hours, the inhibition zones were found around these wells which were measured and compared with the zones made around the standard antibiotic used.

DPPH radical scavenging activity assay: The free radical scavenging activity of the fractions was measured *In vitro* by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier (Ahmad *et al*., 2008). The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (10 - 500 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

$$\text{Scavenging effect \%} = \frac{\text{Control absorbance - Sample absorbance}}{\text{Control absorbance}} \times 100$$

Phytochemical screening: Phytochemical screening of crude extracts/fractions of different parts of our research plants was carried out for the presence of fats, alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, phlobatannins, tannins and terpenoids as per established protocols (Prabhu, 2009).

Results and Discussions

The plant under investigation showed significant biological activities which support the traditional use of the plant to treat various diseases. Therefore this plant species could be an excellent natural source for the treatment of diseases and might be potential targets for the activity guided isolation of its active constituents. The antibactrial profile of the plant leaves exhibited that the crude extract, chloroform and Ethyl acetate fractions showed outsatandinding activities giving above 80% inhibition against *B. anthracis*. The crude extract showed 80% inhibition against *S. dysenteriae*. The Ethyl acetate and crude extarct was also good against *S. typhe* with 78.35% and 76.50% inhibition respectivelly. The *E. coli* being most resistant, non of the fractions of plant leaves was found to be active against *E. coli* (Table 1). The antibacterial activities of plant roots was found to be low as compare to plant leaves, which is probably due to the presence of less phytochemiacls in roots. The crude extract, chloroform and ethyle acetate fractions were found to be potentially active against B. antheracis showing 80.35%, 76.40% and 79.50% inhibition respectivelly. The same three stated fractions were also found active against S. dysenteriae (Table 2). While the crude extract/fractions from the flower of our research plant showed no activity against all the tested bacterial pathogens (Table 3). Crude extracts from nature and compounds purified from these extracts can serve as better drug sources as herbal medicines and have no or minimum side effects, biofriendly and also have benefit due to the combination of medicinal ingredients with vitamins and minerals [Saetung *et al.,* 2005]. Activity guided fractionation and isolation of compounds is the starting point for drug discovery. Bioassays are helpful and simplest tools for testing the activity of plant extracts and on the basis of these activities extracts are preceded for phytochemical studies to isolate novel therapeutic agents (Shinwari *et al*., 2013). Pharmaceutical activities of plant extracts/fractions are due to the presence of major phytocompounds, including terphinoids, fatty acids, carotenes, phenolics, alkaloids, glycosides, flavonoids, tannins (Aqil *et al*., 2006).

Table 1. Bacterial inhibition (in percentage) of crude extract/fractions of *Alhagi maurorum* leaves.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Chloromphenicol
S. typhe	78.35	Nil	69.10	76,50	Nil	Nil	90.35
S. aureus	47.35	25.20	35.30	35.30	15.60	Nil	93.50
V. cholerae	69.45	Nil	35.20	53.20	Nil	Nil	88.30
S. dysenteriae	81.40	52.10	63.50	68.50	Nil	20.30	93.60
E. coli	Nil	Nil	Nil	Nil	Nil	Nil	69.50
B. anthracis	83.40	50.40	80.20	81.10	Nil	Nil	89.40

Table 2. Bacterial inhibition (in percentage) of crude extract/fractions of *Alhagi maurorum* roots.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	n-butanol	Aqueous	Chloromphenicol
S. typhe	63.45	Nil	Nil	61.82	Nil	Nil	86.70
S. aureus	Nil	Nil	Nil	Nil	Nil	Nil	90.65
V. cholerae	55.42	Nil	41.50	47.25	Nil	Nil	89.85
S. dysenteriae	70.57	27.5	58.60	70.20	Nil	Nil	90.30
E. coli	22.5	Nil	Nil	Nil	Nil	Nil	70.45
B. anthracis	80.35	Nil	76.40	79.50	Nil	Nil	87.50

Table 3. Bacterial inhibition (in percentage) of crude extract/fractions of *alhagi maurorum* flowers.

Pathogens	Crude extract	*n*-hexane	Chloroform	Ethyl acetate	*n*-butanol	Aqueous	Chloromphenicol
S. typhe	Nil	Nil	Nil	26.70	Nil	Nil	94.30
S. aureus	20.50	Nil	18.50	Nil	Nil	Nil	90.30
V. cholerae	Nil	Nil	Nil	Nil	Nil	Nil	80.50
S. dysenteriae	Nil	Nil	Nil	Nil	Nil	Nil	95.20
E. coli	Nil	Nil	Nil	Nil	Nil	Nil	70.40
B. anthracis	Nil	Nil	Nil	Nil	Nil	Nil	87.420

Table 4. Antioxidant activities of crude extracts/fractions of leaves, roots and flowers of *Alhagi maurorum*.

Extracts/Fractions	DPPH IC_{50} ± SEM [*m*M]		
	Leaves	Roots	Flowers
Crude	1. 97 ± 0.04	39.53 ± 0.03	25.70 ± 0.05
n-hexane	2.46 ± 0.03	-	-
Chloroform	0.82 ± 0.05	90.03 ± 0.02	-
Ethyl acetate	0.86 ± 0.04	-	-
n-butanol	-	-	-
Aqueous	-	-	-
3-t-butyl-4-hydroxyanisole (BHA)[g)]	0.049 ± 0.03	0.049 ± 0.03	0.049 ± 0.03

BHA, Positive control used in DPPH assays

Table 5. Prelaminary phytochemical profile of *Alhagi maurorum* leaves.

S. #	Phytochemical tests	Methanol extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate fraction	n-butanol fraction	Water fraction
1.	Phenolic compound	+	+	+	+	-	-
2.	Terpenes	+	+	+	+	+	+
3.	Flavonoids	-	+	+	+	-	-
4.	Alkaloid	+	+	+	+	+	+
5.	Saponins	+	+	-	-	-	+
6.	Cardiac glycosides	+	+	+	+	+	+
7.	Anthraquinones	+	+	+	-	-	-
8.	Fats	+	+	+	+	-	-
9.	Coumarins	+	-	+	+	+	-
10.	Phlobatannins	+	+	+	-	-	-
11.	Tannins	+	+	+	-	-	+

+ = Present, - = Absent

In antioxidant tests of different crude extarcts and fractions only the extarct/fractions from leaves of the plant showed free redical scavenging potential which is possibilly due to the presence of phenolic compound in the leaves (Table 4). The leaves are found to be rich sources of phytochemicals as compared to roots and flowers of the plant (Tables 5, 6). The phytochemicals detected in our extracts/fractions are well known for various pharmacological activities. For example alkaloids are common antibacterial, antimalarial, cytotoxic and anticancerous agents (Wirasathien *et al.,* 2006). Similarly saponins have the insecticidal, antibiotic, fungicidal properties. Anthraquinones are antibacterial, antifungal and cytotoxic agents, while terpenoids are antimalarial and antibacterial agents (Kanokmedhakul *et al.,* 2005). Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antineoplastic, antiviral, anti-thrombotic antioxidant and vasodilatory activities. Tannins have shown potential antiviral, antibacterial (Lin *et al.,* 2004) and antioxidant activity (Yokozawa *et al.,* 1998). Fifty-one tannins isolated from oriental medicinal herbs have been evaluated for their antioxidant ability with a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-generating system. The results showed that tannins are potential free-radical scavengers (Yokozawa *et al.,* 1998). Hydrolyzable tannins could cause both double strand and single-strand breakages in DNA (Shirahata *et al.,* 1985). In the past few years, tannins have also been studied for their potential effects against cancer through different mechanisms. Cardiac glycosides have the cytotoxic properties and the Na_K_-ATPase inhibitory properties (Joseph *et al.,* 2005). These compounds are known to have pharmacological activities and therefore are commonly found in medicinal plants.

Table 6. Prelaminary phytochemical profile of *Alhagi maurorum* roots

S.#	Phytochemical tests	Methanol extract	*n*-hexane fraction	Chloroform fraction	Ethyl acetate fraction	*n*-butanol fraction	Water fraction
1.	Phenolic compound	-	-	-	-	-	-
2.	Terpenes	+	+	+	+	-	-
3.	Flavonoids	-	-	-	-	-	-
4.	Alkaloid	+	+	+	+	+	+
5.	Saponins	+	-	+	-	-	+
6.	Cardiac Glycosides	+	+	+	+	+	+
7.	Anthraquinones	+	-	-	-	-	-
8.	fats	+	+	+	+	-	-
9.	Coumarins	+	-	+	+	-	-
10.	Phlobatannins	+	+	-	-	-	-
11.	Tannins	+	-	-	-	-	+

+ = Present, - = Absent

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(Received for publication 30 August 2013)