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Isolation & Identefecation of Glycoside Compound from Alhagi Desert&Alhagi Fields Leavs Extract in Treating Pateint with Renal Stons&Sands

Iqbal jassim Al-Assadi
Chemistry department, College of Sciences, University of Bashar

Abstract
Plants are a source of large amount of drugs comprising to different groups such as (alkaloid, glycoside, saponin, essential oil, bitter principle and, tannins which are useful in the treatment of various diseases such as antispasmodics, emetics, anti-cancer, antimicrobials . The present paper is an Prepare alcohol extract & glycoside extract of Alhagi leaves. We isolated the glycoside compound from this plant.The Isolated compound is shown no antibacterial activity against types of standard strains of bacteria (*Staphylococcus aureus* NCTC 6571 and *Escherichia coli* NCTC 5933). and the cytotoxicity assay against the human red blood cell .The chemical and physical properties were studied by using thin layer chromatography (TLC), IR-spectrum, ultraviolet-visible spectrum, melting point (m.p) and qualitative testes to Isolated glycoside compound The Isolated glycoside compound have been widely used clinically to dissolve urinary stones in the kidney and urinary bladder.
Keywords: Medicinal plants, phytochemicals, extraction Alhagi desert & Alhagi fields kidney stones

Introduction:

Plants are a great source of medicines, especially in traditional medicine, which are useful in the treatment of various diseases (Bako *et al*., 2005). Traditional medicine has not only played a vital role in providing healing but has also contributed to the discovery of most pharmaceutically active substances in plants (Pearce & Puroshothaman, 1992) which have been used in the commercial production of drugs. It has been estimated that, up to 90% of the population in developing countries rely on the use of medicinal plants to meet their primary health care needs (WHO, 2002).

According to (Schippmann *et al*. 2002) more than 50,000 plant species are used for medicinal purposes worldwide, of which almost 13% are flowering plants.

Medicinal plants containing active chemical constituents (alkaloid, glycoside, saponin, essential oil, bitter principle and, tannins) in its parts for example, root, stem leaves, bark, fruit and seeds, which produces a definite curing physiological response in the treatment of various ailments in humans and other animals (Adhikari *et al.,* 2010).

Due to easy availability, no side -effects and sometimes only source of health care, the demand for medicinal plants is increasing in both developing and developed countries.

The family Leguminosae is comprised of about 550 genera and more than 13,000 species (Bolus, 2000), including several members that are used in folklore medicine (Lewis & Lewis 1977).

Chemical investigation of the Alhagi species revealed the presence of several contents such as fatty acids and sterols (Ghosal *et al*., 1974; Kudliki etal., 1991; Kalhoro *et al*., 1997), vitamins and Twelve flavonoids (AlYahya *et al*., 1987; El-Saayed *et al*., 1993; Singh *et al*., 1999), coumarnine and alkaloid (Behari & Gupt, 1980), were isolated from Alhagi graecorum Boiss.

These flavonoids were identified as tamarixtin 3-O-dirhamnoside, isorhamnetin 3-O-glucosylneo-hesperidoside, isorhamnetine 3-O-robinoside, isorhamnetin 3-O- rotinoside, quercetin 3-O-rhamnoside, kampferol 3-O-galactoside, quercetin 3, 7-diglycoside, isorhamnetin 3-rutinoside, daidzein 7, 4 - dihydroxyisoflavone, calycisin 3-hydroxyformononetin, and isorhamnetin and tamarxtin aglycones.

Alhagi maurorum Boiss is customarily used in folk medicine as a remedy for rheumatic pains, bilharziasis, liver disorders, and for various types of gastro intestinal discomfort (Bolus, 1983), but there is no scientific background that supports this use .

Alhagi camelorum (camel thorn) is an invasive, peren- nial shrub in the Legume family with fairly deep creep- ing root, 60 to 100 cm long. It is used in Iranian herbal medicine and is known for its antiasthmatic, aphrodiasiac, antipyretic, diuretic, expectorant and laxative effects (Ahmad *et al*, 2009),

One of the medicinal plants in Iraqi folic medicine is Alhagi graecorum Bioss-Maurorum MEDI (Leguminosae(Akool in Iraq, Camel Thorn, Persian Manna plant) (Nadhal etal 2010). It's used for rheumatic pains, liver disorders, urinary tract infection and for various types of gastrointestinal discomfort.(Jehti *et al* 1983)

Kidney stones are small, hard deposits of mineral and acid salts developed from crystals that separate from the urine within the urinary tract. The most common type of stone contains calcium in combination with either oxalate or phosphate (Tyagi *et al*. 2012)

The treatment of urolithiasis is mainly considered with the dissolution of existing stones and preventing the reoccurrence of stones. Standard pharmaceutical drugs used to prevent and cure urolithiasis are not effective in all cases, costly, quite common reoccurrences, risks of long term fertility, potential side effects and no guarantee . (Lipismita et al 2011)

All parts of the plant drink, or incense. Urinary stone occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10 % in which there is a predominance of men over women that can be observed with an incidence peak between the fourth and fifth decade of life. Reoccurrence of stone formation is a common part of the medical care of patients with stone disease1. These stones may be classified on the basis of their constituent i.e. Calcium-containing stones, specially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% , magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1% . Out of all the types most common type is calcium oxalate ormagnesium ammonium phosphate type which generallyoccurs commonly. (Otnes1983) (Williams &Wandzilak1989). Many medications and remedies have been used during the past many years to treat urinary stones.(Narayana et a1967) . (Bahl a&Seshadri 1970)

Materials & Methods

Plant Materials:

Leaves of Alhagi desert & Alhagi fields was collected from al-Zober city in Basra in September 2014 . The plant was deposited in the Herbarium of Basra at Collage of Science / University of Basra. The leaves was dried at 40 c then ground by blender (Rotel coffee grinder type 24)and kept in nylon bag s until the day of used .

Preparation of Alcohol Extract of Alhagi desert & Alhagi fields leaves

Twenty-five grams of ground leaves powder were refluxed with (250 ml) (70% EtOH-water) (Iraq medical alcohol 96%) for (12 hr.), then cooled and filtered. The solvent was dried and concentrated by using Rotary evaporator (PUCHI Rotavpor-RE, Switzerland) at (50ºC). The dryness of the extract was completed by using oven at 50ºC) yielding black green powder (1,1.5,1.9,2.2)gm Alhagi fields leave& , Alhagi desert leaves, respectively . (Pieroni *et al*., 1996)

Preparation of Glycoside extracts

Fifty grams of ground leaves powder was refluxed with (300 ml) of 2% Acetic acid for (8hr) then cooled and filtered. The solvent was dried and concentrated by using Rotary evaporator (PUCHI Rotavpor-RE,Switzer) (Yan-bo *et al* ., 2012)

Preliminary qualitative Chemical Test

The chemical family of the extracts & isolated compound was implemented using several tests such as: Phenolic compound(Harbone,1993). Tanin (Harbone,1993) . Flavonides (Lima *et al* ., 2010) carbohydrates (Rajendra 2011) proteins(Sadaalla,1981) ,free amino acid (Harbone,1993) Glycosides (Al-Kazaraji,1991) alkaloids(Liliwiranis *et al* ., 2011) saponine (Sawant & Godghate,2013)

Thin layer chromatography (TLC):

To determine the purity and relative to front (R_f) of glycoside extract , a thin layer chromatography was carried out for (90min). On silica gel plates (2x9cm) in a pre saturated chamber of the mixture of water :methanol: ethyl acetate (5:2:5)the plate were dried and the spot which appeared were developed with UV-lamp at (336nm), iodine vapor (Harbone 1993)

Column Chromatography

Column chromatography was performed on a classic 20 cm long ×2 cm diameter glass column packed with silica gel). The methanol solution of the extract (20 mL) was applied to the column by use of a pipette and the column was eluted with of (water :methanol:ethyl acetate (5:2:5)) . reagent. The fractions were evaporated to dryness

Determination of melting point:

Melting point electro-thermal is used for the determination of melting point of the isolated compounds.

Spectroscopy:

a- Inferred spectrum: FT-IR spectrum of the isolated compound was recorded with (FTIR8400S SHIMADZU–Japan in the college of Science, Chemistry Department, University of Basrah.

b- Ultraviolet and visible spectra: ultraviolet and visible spectrum of the isolated compound was carried out in the College of Science, Department of Chemisty, by using ethanol as the solvent, and the spectrum recorded with the (BECKMAN-COULTERDU530-Life Science UV/VISIBLE Spectrophotometer).

Determination of the antibacterial activity:

A filter disk assay was used to determine the antibacterial activity of the isolated compound (30,000 µgm/ml) against types of reference strains of gram positive and gram negative bacteria (Staphylococcu aureus NCTC 6571 and Escherichia coli NCTC 5933) which are tested using plate of Muller-Hinton agar. The antibacterial activity was defined as the clear zone of growth inhibition [11]. The minimum concentration (MIC) of the

isolated compound was estimated against types of reference strains of gram positive and negative bacteria with different concentration of the isolated compound ranging from (1-1000 µgm/ml) (Collee, *et al* 1996).

Cytotoxicity assay:

The cytotoxicity activity of the isolated alkaloid & complex were determined against human red blood cells .Different concentration of the compound were prepared separately dissolved in DMSO solution ,then 100 of each concentration was add to 2ml .of blood .The turbidity of the mixture was examined after 30 10 and 60 min before the blood cells were heamolysate completely\ (Nair *et al* 1989)

Determination of kidney stones component :

The stone was collected from different pateint then wash with n-saline and dried at 30 c then ground by mortar and then analysis the organic and inorganic compound (Curhan *et al* 1998)

Antiurolithiatic Effects of kidney stone

In three beaker put equal component from buffer solation P^H 3.7 & Stone powder in the first beaker put 1gm from Alhagi desert in second beaker put 1gm of Alhagi fields & in third beaker put buffer solution only then incubated in 37 c° with starrier.

Result & dissection :

The results of preliminary qualitative test shown in a table (1), where the appearance of Alhagi desert , Alhagi fields leaves , and isolated compound from glycoside family, which then gave a negative result for each of carbohydrate, glycoside, saponin, alkaloid testes. The early search appeared the seed of . Alhagi greacoum contains a number of active constituents like glycoside.

Thin layer chromatography study for isolated compound give only one spot using different solvent system and different types of (TLC) plates by using some reagent as a developer for this spot, shown in a table (2), relative of front (R_f) equal (0.5,0,8), it is organic compound have conjugated double bonds and phenol group.

Melting point (m.p), was also tested and it was found that the isolated compound has sharp melting point (126-128ºC), which means the isolated compound is pure.

The FT-IR spectrum for the isolated compound is shown in table (3), the appearance of a single broad peak at (3138cm-1) related to the vibration stretching for (OH) bond indicated the presence of phenol group. The band at (1755 cm-1) is related to the vibration stretching for(C=O) bond of carbonyl group. The band at (3045 cm) is due to the vibration stretching for ring bond of benzene ring in aromatic compound. The band at (2808-2870cm-1)is due to (C-H) aliphatic .The band at (1080 cm-1) is due to the vibration stretching for (C-O) bond of ether. And the band at (1402 cm-1) is related to the vibration biniding for (C-H) bond of benzene ring. [Silverstein , 2010]

The ultraviolet-visible spectrum, shown one peak at λ equal to(268nm)), due to presence the pairs of electrons $\pi - \pi^*$ max (Schieber *et al* ., 2003)

The antibacterial activity of isolated compound was determined by using filter disk assay. The results, show that the isolated compound has not good antibacterial activity against gram positive and gram negative bacteria: which are (*Staphylococcus aureus NCTC 6571 and Escherichia coli NCTC 5933).*

Cytotoxicity assay: The result show that the cytotoxicity activity of the isolated compound was determined against human red blood cells .Different concentration of the compound they are no hemolytic effect on blood cell (Joy ,et al.,1998), (Roberson 2008)

Table (1) the qualitative chemical analysis for the isolated compound & alcoholic extract of

Compounds\reagent	Leaves of Alhagi desert	Root of Alhagi desert	Leaves of Alhagi fields	Root of Alhagi fields	Glycoside
Dragendroff's	+	+	+	+	-
Wagner's	+	+	+	+	-
Molisch's	+	+	+	-	+
Tannin's	-	-	-	-	-
Ninhydrin	+	-	+	-	-
Glycosides	+	++	+	+	++
Saponins	-	+	-	+	-
Libermann Burchard's	+	+	+	+	-
Ferric Chloride	+	-	+	-	-
Flavonoids	+	-	+	-	-

Table (2) Thin layer chromatography, R_f values for the isolated compound from Alhagi desert& Alhagi field glycoside

Solvent systems	Developers	Number of spot	Rf values Alhagi desert glycoside	Rf values Alhagi fields glycoside
Water –Methanol-Ethyl acetate 5:2:5	The eyes	2	0.5 , 0.8	0.56,0.95
	I2- Vapor	2	0.5 ,0.8	0.56,0.95
	UV-lamp (366nm)	2	0.5,0.8	0.56,0.95

Table (3) the infrared absorption peak and their related functional group for the isolate glycoside from Alhagi desert leaves

Range(cm^{-1})& intensity	Bond shape	Bond	Functional group
3138	St	O-H Alcohole	Hydrogen bonded O-H Stretching
3045		C-H of heterocyclic	C-H Stretching
2856		-CH_2 in alphatic compounds	C-H Stretching
1730		C=O Carboxylic acid	C=O Stretching
1629		C=C in conjugated system	C=C Stretching
1402		C-H in heterocyclic	C-H bending
1080		C-O in Carboxylic acid	C-O Stretching
1076		C-O in alcohol	C-O Stretching

Table (4) Loss in weight of kidney stone in different time

Time \hour	T1 gm	T2 gm	T3 gm
0	0.192	0.231	0.229
1	0.168	0.195	0.226
4	0.131	0.176	0.224
5	0.128	0.169	0.221
6	0.126	0.164	0.221
7	0.113	0.158	0.220
8	0.074	0.145	0.218
Total weight loss	0.118	0.086	0.01

When T1 was glycoside of Alhagi desert ,T2 was glycoside of Alhagi fields And T3 was control

The active compound in both types of Alhagi is glycoside. Herbal agents act by allowing spontaneous passage of small calculi in urine by increasing the urinary volume, P^H.

The herbs also act by regulating oxalate metabolism, by maintaining balance between inhibitors and promoters of crystallization, by producing anti-oxidant, anti-microbial, analgesic, anti-inflammatory activities. Modern medicines are proved to target only one aspect of urolithiatic pathophysiology whereas herbal remedies have been shown to exert effectiveness at different stages of stone pathophysiology. . (Joy *et al.* 2012)

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