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# Antibacterial activities and phytochemical analysis of leafextracts of *Iphionascabra*plant used as traditional medicines in ALKHUMS-LIBYA

**Afifa Milad Omeman**
Department of Chemistry, Faculty of Education/Elmergib University
Omemanafifa@Gmail.com

**Abstract:** Medicinal plants contain inherent active ingredients used to cure diseases or relieve pain and traditional remedies made from these plants play an important role in maintaining the health of 70%–80% of people.The present study aimed to determine in vitro antibacterial activities and phytochemical analysis of *Iphionascabra*Called boveawhich are commonly used as traditional medicine in Libya. Leaf extracts of *plant* were prepared using solvents, ethanol and water,aqueous and ethanol extracts leaf of *Iphionascabra*revealed presence of major groups of phytochemical bioactive constituents namely alkaloids, diterpenes, flavonoid, tannin, phenol,Steroid,Coumarins and Glycosides .However, the saponins were absent in aqueous extract and presence in ethanol extract.furthermore, the quantities analysis of ethanolic extract of leaves had significant amount of chemical compounds 90% followed by aqueous extract of leaves 84%. Additionally, the crude extracts of *Iphionascabra*were tested (using the Disc Diffusion Method) for their antimicrobial activity against the bacterial pathogens. The influences of aqueous and ethanol extracts on some pathogenic: one strains of gram-positive bacteria include *Staphylococcus* and one strains of gram-negative bacteria including *Shigella*. The results showed that the both extracts *Iphionascabra* leaves have antibacterial activity in a broad-spread way and able to inhibit strongly the growth of *staphylococcus* and *Shigella*.So, *Iphionascabra* is a good source phytochemicals and can be used as a medicinal herb.

**Keywords:** *Iphionascabra,* Soxhlet Extraction, Phytochemicals analysis, Antibacterial Activities

## Introduction

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Savithramma, 2012).The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and the plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effect and can be natural composite sources that can be used as anti-infective agents. Plants to be exploited for medicinal purposes have to undergo basic phytochemical screening as the first step toward the ultimate development of natural drugs (Saxena, 2010).

*Iphionascabra* belonging to family Compositae (Astraceae), *Iphiona* is a small genus of about eleven species, which is distributed from North-East Africa to central Asia (Anderberg, 1985). Studies on *Iphionascabra* revealed that polysulphatedflavonoids and sesquiterpene glycosides were the major constituents and seem to be characteristic for this genus (Ahmed and Mabry, 1987; Ahmed, 1988), andthey are rich in coumarin and pyrrolizidine alkaloid.

*Iphionascabra* is used in traditional medicine as an antispasmodic drug (Font-Quer, 1990), this herb has a strong aromatic odour and it is rich in essential oils containing of carminative agents such as comphor, borneol , intermedeol and organic acids such as caffeic acid (Guillén, 1996), it has been consumed as infusions for a long time and popularly known as rock tea in Spain, it has been consumed as infusions for a long time and popularly known as rock tea in Spain (Pardo , 2005). The results indicate selective antineoplastic activity toward human colon carcinoma cells, the exploratory studies of some pharmacological properties belonging to Iphionascabra extracts proved that the extracts have anti-inflammatory, anticoagulant and hypotensive effects.

**Materials and Methods**

***Collectionand identifications and Sample preparation of plants***

Fresh leaves samples of *Iphionascabra*were identified for its ethno-medicinal uses and were collected in September, 2022 from naturally growing located in Alkhums in Libya. The samples were identified at the herbarium section of the Department of Biological Sciences, Faculty of Science El-Mergib University Alkhums. Libya.

The Leaves of plant were cleaned and shade dried at room temperature for 7 days. The dried plants were pulverized by an electrical blender and passed through the 20 µ mesh sieve and stored at room temperature in closed containers in the dark until used.

**Preparation of Plant Extract**

The phytochemical extraction was performed usingwater and organic solvent (ethanol 70%) extraction,the extraction was performed by Soxhlet extraction method. This extraction was done by taking 10 gof dried plant powder and was placed into a glass thimble then extracted with 250 ml of different solvents separately (ethanol and water). The extraction processes carry on till the solvent in siphon tube of Soxhlet apparatus become colorless. After that the extract was heated on rotatory vacuum evaporator at 35°C until all the solvent evaporated. The concentrated extracts were kept in refrigerator at $4^{o}$C until further phytochemical screening.(Kaleeswaran, 2010).

The yield of the extracts was calculated using the following equation:

R% = (Me / M v) * 100

R% = Production productivity of extracts %

Me = Mass of dry plant material extracted after solvent evaporation.

Mv = Dry plant material mass used for extraction.

**PH measurement of the extracts**

In a 100 mL flask, placed 2g of the dry extracts and diluted with 50ml of distilled water. By using pH meter (HANNA Instruments) at 25 °C. Results as shown in table

**Qualitative phytochemical screening**

Qualitative phytochemical screeningof plant extracts was carried out for the identification of various classes of active chemical constituents like alkaloids, flavonoids,Proteins and amino acids,Steroid, tannins, phenolic compounds, saponins, andterpenoids using different methods described. In general, test for the presence or absence of phytochemical compounds using standard methods involves the addition of an appropriate chemical agent to all the extracts in a test tube and shaken by using the standard following methods.(Raaman, 2006), (Rahul, 2010), (Ajayi, 2011), (Ramasamy , 2012), (Jaradat, 2015).

**alkaloids**

a. Dragendorff test: To 1 ml of extract, a few drops of Dragendorff's reagent were added. A prominent yellow precipitate indicates a positive test.

b. Wagner test: A few drops of Wagner's reagent were added by the side of the test tube to 1 ml of extract. A reddish brown precipitate confirms the test as positive.

**Tannins and phenolic compounds**

Ferric chloride test: One milliliter of extract was separately stirred with 10 ml of distilled water and then filtered. A few drops of 5 percent $FeCl_3$ were added to the filtrate. Blue-black or blue-green coloration or precipitation was taken as an indication of the presence of phenols and tannins.

**Flavonoids**

Alkaline reagent test : A few drops of a 20 percent NaOH solution was added to 1 mL of extract. When HCl is added, the yellow color of the extract turns to a colorless solution that indicates the presence of flavonoids.

**Saponins**

Foam test, about 1 ml of the sample extract was boiled in 20 ml of distilled water in a water bath and filtered; 10 ml of the filtrate was mixed with 5 ml of distilled water and mixed vigorously for 15 minutes to form a stable persistent froth. The presence of froth after 5 minutes was taken as an indication of the presence of saponins.

**Proteins and aminoacids**

The extracts5 ml were added 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

**diterpenes**

The extracts (5 ml) were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes**.**

**Carbohydrates**

a. Molisch's test: 2ml of Molisch's reagent was mixed with 5ml of Crude extract and the mixture was shaken accurately. Afterwards, 2ml of concentrated $H_2SO_4$ was poured prudently along the sideof the test tube. The appearance of a violet ring at the interphase indicated the presence of carbohydrate**.**

b. Benedict's test: 2ml of Benedict's reagent was mixed with 5ml of crude extract and boiled; a reddish brown precipitate formed which indicated the presence of the carbohydrates.

**Steroid**

The extracts (5 ml) were dissolved in 10 ml of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish color formed at the interphase indicates the presence of a steroid ring.

**Coumarins**

0.5 g of the moistened various extracts was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1 N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

**Glycosides**

Five ml each of various extract were hydrolysed separately with 5 ml each of conc. HCl and boiled for few hours on a water bath and hydrolysates were subjected to the following test: A small amount of alcoholic extract of samples was dissolved in 1ml water and then aqueous 10% sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

**Antibacterial activity**

The antibacterial activity of the extractswas determined by the disc diffusion method

**Microbial strains**

two types of pathogenic bacteria (one Gram-positive *Staphylococcus*andone Gram-negative*Shigella*) were used against crude plant extracts.

**Disc diffusion method**

1. Firstly prepared the extract disc by using What man No-1 filter paper with the help of punching machine and then autoclaved.
2. Soaked the discs in already prepared different concentrations of extracts and left overnight.
3. prepared the petri-plates with suitable agar media (already prepared and autoclaved).
4. Spread the bacterial strains on their respective agar media.
5. Test extract loaded disc were placed on respective bacterial and then incubated atsuitable temperature i.e. 37˚C for bacteria
6. After incubation period, the zone of inhibition was measured and recorded.

**RESULTS AND DISCUSSION**

As shown in Table 1 the percentage yields of each chemical constituent's present in *Iphionascabra* leaves were 84% and 90% of aqueous and ethanolic extractsrespectively. As we as, the aqueous extract of leaves showed highest pH range of 6.99 compare ethanol extract 6.05.

***Table 1: Results of pH values and per cent yields ofIphionascabra***

| Name s' plant | Name of part | Percentage Yield (%) | | pH Value |
|---|---|---|---|---|
| | | Chemical Constituents | | |
| *Iphionascabra* | leaves | aqueous extract | 84. 331% | 6.99 |
| | | Ethanolic extract | 90.670 % | 6.05 |

**Qualitative analysis**

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, saponinsetc. It is further observed from Table 2 that the aqueous leaf extract of *Iphionascabra* depicted the presence of alkaloids, diterpenes, flavonoid, tannin, phenol, Steroid, Coumarins, carbohydrates, Glycosides, Proteins and amino acids, while negative results were obtained for saponins. On the other hand, the leaf extract in ethanol exhibited positive results for all the phytochemicals. The aqueous leaf extract depicted results similar to that of ethanolic extract for all the phytochemicals except saponins.Phytochemical compounds such as alkaloids, saponins, tannins, flavonoids and steroids have been known to be biologicallyactive and thus partially responsible for the antimicrobial activities of plants, hence their use in traditional medicine. The presence of Phytochemical compounds in crude extract is important since they have been reported to exhibit antimicrobial, anti-inflammatory, analgesic, anti-allergic, antioxidant, antitrypanosomal and antileishmanial properties.

Finally, it may be concluded that the phytochemical screening of *Iphionascabra* in this studyrevealed the presence of all the phytochemicals except saponins in aqueous leaf extracts. The major phytochemicals are present in ethanol extract than aqueous extract, therefore, the ethanolic extract is showing better result compared to aqueous extract. Results of preliminary screening of *Iphionascabra* plant extracts are shown in Table 2.

***Table 2: Phytochemicals analysis in the leaves extracted byIphionascabra***

| **Chemical Component** | **Crude Extracts of Leaves of *Iphionascabra*** | | **The Tests Names and Resulted in Colours** |
|---|---|---|---|
| | **aqueous extract** | **Ethanolic extract** | |
| Alkaloids | + | ++ | Dragendorff: Reddish-Brown Precipitate |
| | ++ | +++ | Wagner: Reddish-Brown Precipitate |
| Tannins &Phenols | ++ | ++ | Ferric Chloride: Blue- Green or Black |
| Flavonoids | ++ | ++ | Alkaline Reagent: (Sodium Hydroxide test): yellow colour |
| Saponins | - | + | Foam:Persistent foam |
| Proteins and amino acids | + | + | Ninhydrin: Violet |
| Diterpenes | ++ | ++ | copper acetate test: green color |
| carbohydrates | + | + | Mulish: Violet Ring |
| | - | + | Benedict: Reddish Brown Precipitate |
| Steroid | + | ++ | Chloroform: Red |
| Coumarins | + | + | NaOH: yellow fluorescence |
| Glycosides | + | ++ | conc. HCl, 10% sodium hydroxide:yellow colour |

+ = low concentration, ++ = moderate concentration, +++ = high concentration, - = absent.

Table3: Antibacterial activity of aqueous and ethanol extract of *Iphionascabra*

| Human Pathogenic Bacteria | Zone of Inhibition (in mm.) *Iphionascabra* | |
|---|---|---|
| | Aqueous | Ethanol |
| *Staphylococcus* | 13mm | 16mm |
| *Shigella* | 15mm | 19mm |

**Antimicrobial Activity**

The results are presented in Table 3 of antibacterial activity of*Iphionascabra*extracts which were analyzed against specific of humane pathogenic bacteria andone of bacterial isolates used in this study were chosen because they are associated with gastrointestinal infections, were the maximum antibacterial activities was observed in ethanol extract of*Iphionascabra*19mm against *Shigella* then 16mm against *Staphylococcus*, followed by 15mm formed from aqueous extractagainst *Shigella*and 13mm was formed from the aqueous extract of *Iphionascabra*against*staphylococcus*. Overall results, this study confirmed that *Iphionascabra* extracts have great potential as traditional medicines.

**Conclusion**

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine, The aqueous and ethanolic extracts of *Iphionascabra* exhibit varying degrees ofanti-bacterial activities against two bacterial species namely *staphylococcus* and *Shigella*. In conclusion, phytochemical analysis and Antibacterial shown by this plant provides a scientific basis and thus, validates their uses as traditional medicines. To the best of our knowledge this is the first paper about phytochemical analysis and Antibacterial Activities of *Iphionascabra* from libya.However, further studies are necessary to elucidate the compounds responsible for this activity to valorize its pharmaceutical uses.

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