Anti-Microbial Activity of Ethanolic and Aqueous Extract of *Cynanchum acutum*

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Abstract: *Cynanchum acutum* is an important medicinal plant belonging to the family of Apocynaceae. It is a well recognized plant in the traditional medicine and is used by people in rural areas. This study is aimed to evaluate the anti-microbial effects of ethanolic extract of *Cynanchum acutum*. The antimicrobial property of the *Cynanchum acutum* was studied against gram positive and gram negative microorganisms using the agar disc diffusion method, in which ethanolic extract of *Cynanchum acutum* has shown bigger zone of inhibition than aqueous extract. These results demonstrate that *Cynanchum acutum* possesses anti-microbial effects and it can be a good candidate for producing of anti-microbial drugs.

Keywords: Anti-microbial, aqueous extract, *Cynanchum acutum*, ethanolic

INTRODUCTION

According to world health organization 1 of the main causes of morbidity and mortality is diseases resulted from pathogenic bacteria and fungi (World health organization, 1998). On the other hand the increasing resistance of pathogens to drugs encourages scientists to search for new antimicrobial sources like medicinal plants (Karaman et al., 2003). Owing to less side effects and economical reasons plant-based drugs have got great attentions during past few decades. Many commonly used drugs are of herbal origins and over 80% of world’s people use medicinal plants for their primary health care needs. The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition. The search for agents to cure infectious diseases began long before people were aware of the existence of microbes. These early attempts used natural substances, usually native plants or their extracts and many of these herbal remedies proved successful (Sofowora, 1982). The effective substances of many plant species are isolated for direct use as drugs, lead compounds or pharmacological agents (Fabricant and Farnsworth, 2001). Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Karthikeyan et al., 2009; Lozoya and Lozoya, 1989). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc (Gordon and David, 2001). Many plants possess antimicrobial activities and are used for the treatment of different diseases (Arora and Kaur, 1999). *Cynanchum acutum* is an invasive plant species belonging to the family Apocynaceae. It has been used as a purgative in the French pharmaceutical Codex (Garnier et al., 1961) and its milky latex is used for skin and eye problems in Tunisian folk medicine (Sayed et al., 2003; Boukef, 1986) and its seeds are edible in some parts of Iran. The photochemical investigations on *Cynanchum acutum* L. have revealed the presence of several natural compounds including β-sitosterol, lupeol, lupyl acetate and α-amyrin (Halim et al., 1990), sarcostine, quercetin and quercetin 3-O-β-D-galactoside (El Sayed et al., 1994), four flavonoid glycosides: quercetin di-O-hexoside, quercetin 3-O-rhamnosyl (1→2) glycoside, quercetin 3-O-galactoside and quercetin 3-O-xiloside (Heneidak et al., 2006) and 2 simple coumarins: scopoletin and scoparone (El-Demerdash et al., 2009) as well as of 7 other flavonoids (Ghada et al., 2008). Studies on other species of this genus which have close affinities with *C. acutum* also have been done and the following products have been distinguished: steroidal glycosides (Liu et al., 2007), carbohydrates (Yi-Bin et al., 2004), alkaloids (Tian-Ying et al., 2001), phenolic compounds (Lou et al., 1993) and triterpenes (Konda et al., 1990). This close affiliation enables us to predict the presence of these products in *C. acutum*, since close genotypes ends to production of similar compounds. Antidiabetic and antioxidant activity of
metanolic extract of aerial parts of *Cynancum acutum* has been reported and antiulcerogenic effects of ethanolic extract of the plant also have been shown in rats (Atta et al., 2005). The current study was undertaken to evaluate the anti-microbial activity of ethanolic and aqueous extract of *Cynancum acutum* by, till now no pharmacological evaluation has been done on the anti-microbial activity of *Cynancum acutum*.

**MATERIALS AND METHODS**

**Plant material:** *Cynancum acutum* was collected from Sistan and Baluchestan province (Iran) in March 2011 and authenticated at Medicinal Plants and Drugs Research Institute, Shahid-Beheshti University, Tehran, Iran. Its leaves and fruits were dried, under shade and powdered.

**Preparation of the ethanolic and aqueous extract of *Cynancum acutum***: Air dried powdered plant was divided in to 2 equal parts (1 kg each); one part was macerated with ethanol (90% v/v) in glass percolator and allowed to stand at room temperature for about 24 h. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The percentage yield was 19.6%, 2nd part of powdered was cold macerated with distilled water then the same procedure was repeated as mentioned above. The percentage yield was 10.5%.

**Phytochemical screening:** The freshly prepared ethanolic and aqueous extract of *Cynancum acutum* was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer’s, Hager’s and Dragendorff’s reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish’s, Fehling’s and Benedict’s reagent; proteins and amino acids with Millon’s, Biuret and xanthoprotein test. Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red; Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures (Trease and Evans, 1989). These were identified by characteristic color changes using standard procedures. The screening results were as follows: Alkaloids +ve; Carbohydrates +ve; Proteins and amino acids +ve; Steroids -ve; Sterols +ve; Phenols +ve; Flavonoids +ve; Gums and mucilage +ve; Glycosides +ve; Saponins -ve; Terpenes +ve and Tannins +ve. Where +ve and -ve indicates the presence and absence of compounds.

**Microorganisms:** Bacterial (Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Poreus vulgaris) were procured from Pastore Diagnostic Labs, Tehran, Iran.

**Chemicals and drugs:** Ethanol was obtained from Bidestan Co. (Tehran, Iran). Cloxicillin, Amoxiclav, Cefuroxime, Cefixim were from Tablets Iran P.vt, Limited (Tehran, Iran) were used as Reference Antibiotics (RA) against bacteria. The nutrient agar was from Zist Iran (Tehran, Iran). Indomethacin was from Merck.

**Anti microbial activity:**

**Sensitivity test: agar disc diffusion assay:** The disc diffusion method was followed to evaluate anti-microbial activities using a range of microorganisms. Sterile Discs (Whitman, 6 mm) were impregnated with 10 μL of reconstituted crude extracts (1 mg/mL) and placed on the surface of Muller-Hilton agar dispersion plates inoculated with microbes. Each extract was tested in triplicate. Control discs contained pure DMSO (100%). Standard antibiotics, Cloxicillin, Amoxiclav, Cefuroxime and Cefixime (30 μg disc-1), were used to eliminate variation between plates. Agar plates containing bacteria were incubated at 37°C for 24 h. Inhibition zones were recorded as the diameter of growth-free Zones (IZ), including the diameter of the discs, in mm, at the end of the incubation period (Salie et al., 1996).

**Statistical analysis:** All values are expressed as mean±SEM. Data were analyzed by non-parametric ANOVA followed by Dennett’s multiple comparison tests and other data was evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

**RESULTS**

The ethanolic and aqueous extracts from *Cynancum acutum* has shown inhibition effects on the growth of all the organisms tested, but their efficiency in inhibition was varied from one organism to another. In almost all, the tested organisms’ growth was inhibited by both ethanolic and aqueous extract has shown higher range of Inhibition Diameter (IDZ) from 08 to 19 mm, where as ethanolic extract has shown inhibition range of 15-20 mm. Staphylococcus aureus is more sensitive and Escherichia coli is least sensitive to ethanolic extract. Whereas aqueous extract has shown inhibition range of 08-19 mm. Bacillus subtilis is more sensitive and Escherichia coli are least sensitive to aqueous extract. Cloxicillin, Amoxiclav, Cefuroxime, Cefixime ranged from 20-28 mm at a concentration of 30 μg/zone. All IZD corresponding to test organisms are tabulated in Table 1.
Standard mechanisms.

Cellular experiments are warranted to explore its action evidence for folk uses of it. Further molecular and inflammatory effects, which provide pharmacological Cynanchum acutum these extracts. In conclusion, our results indicate that active principles will confirm this hypothesis and provide more explanation on mechanism of action of the tested extracts. However, the isolation of the presence of antimicrobially active metabolites classes such as flavonoids, phenols, terpenoids, alkaloids, glycosides might explain the wide spectrum of activity of the tested extracts. However, the isolation of the active principles will confirm this hypothesis and provide more explanation on mechanism of action of these extracts. In conclusion, our results indicate that Cynanchum acutum has anti-microbial and anti-inflammatory effects, which provide pharmacological evidence for folk uses of it. Further molecular and cellular experiments are warranted to explore its action mechanisms.

**REFERENCES**


