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Efficiency of aqueous and alcoholic extract of *Costus speciosus* roots on Iraqi pneumonic isolated bacteria

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ABSTRACT

The decorative, pharmaceutical plant, *Costus speciosus* can be useful for health as therapeutic agent. This study tends to estimate the antibacterial activity of this plant rhizomes (aqueous and methanol extract) on *Pseudomonas aeruginosa* growth using the well diffusion method. In this study, the *C. speciosus* sundried rhizomes were powdered and extracted using Soxhlet apparatus, diluted for specific concentrations of the collected dried extract, then qualitative detection in raw extract of the plant rhizomes was performed for detection of the active compound's presence. The *P. aeruginosa* which is pathogenic bacteria, was placed in a culture of brain heart infusion broth and adjusted to McFarland tube. The resulted extract was two kinds (three samples for each concentration), initial concentration was adjusted to 200 mg mL^{-1}. Measurement of zones of inhibition were performed after incubation, followed by statistical collection and analysis of data. Five compounds in the *C. speciosus* rhizomes methanol extract were present using the qualitative detection of the active compounds including alkaloids, saponites, turbines, tannins, and flavonoids. The extracts showed influential inhibition of growth of bacterium used in this study. The methanol and aqueous extracts with the concentration of 200 µg mL^{-1} showed the maximum antibacterial effect with the zone inhibition diameters of 16 and 17 mm respectively. The methanol extract was moderately effective with the concentration of 100 µg mL^{-1} and the inhibition zone diameter of about 10 mm.

Key words: *Costus speciosus*, Methanolic extract, Antibacterial.
Article type: Research Article.

INTRODUCTION

For a long time, there was increasing interest in extracting drugs from herbs to use them as alternative therapy. Herbal drugs are safer, less expensive and with less side effects compared to synthetic drugs (Hasan & Qari 2010). *Costus speciosus,* which is a decorative and pharmaceutical plant, contains different components, so it was a goal for many studies among other herbals. Many studies reported the antifungal and antibacterial properties of the plant *in vitro* (Aritharan *et al.* 2012; Manorama *et al.* 2013). The essential oil in its roots is used as a beneficial drug for many disorders, e.g., chronic bronchitis, common cold, asthma, sore throat, diarrhoea, obesity, burns and wounds treatment, heart diseases and atherosclerosis etc. (Joji & Benna 2010). The diagnosis of these compounds led to extensive studies to estimate their effects (Muniyandi *et al.* 2013). *Pseudomonas aeruginosa*, which is a common cause of the nosocomial infections, is an opportunistic pathogen present commonly in the environment especially in soil and water, but is also found on plants and sometimes animals, and humans. This bacterium is highly resistant to antibiotics and can grow in various generally-inhospitable environments, mostly by its virulent factors, e.g., the ability to form resilient biofilms. Therefore, the aim of this study was to detect the antibacterial activity of aqueous and methanol extract of *C. speciosus* rhizomes on *P. aeruginosa* during *in vitro* condition. Medicinal plants are very important in human culture to satisfy the primary health care needs. In developing countries many individuals use medicinal plants as traditional drugs. According to WHO (World Health

Organization), up to 80% of people in the world depend on traditional medicinal system for some problems of primary health care (Hasan & Qari 2010). Medicinal plants produce numerous compounds that have a known therapeutic property. In the last few decades, there was an increasing interest in deriving drugs from plants to control diseases. We should keep in mind that the herbal products are safer than synthetic products which may have side effects to the human and environment (Pawar & Pawar 2014). India is a rich country in indigenous herbal resources. There are 2500 out of 20,000 plant species, useful medically. Rich diversity and traditional knowledge, push the world to look towards India to develop new natural, safe, herbal drugs to treat different diseases (Karthikeyan *et al.* 2012).

Costus speciosus

The agro-climatic conditions in India provide an ideal environment for the growth of medicinal plants. Between different plants studied, *Costus speciosus* is an important plant traditionally, medicinally and pharmacologically. There are over 100 species of the *Costus*. The different species of *Costus* differ in the colour of its flower such as *C. barbatus, C. chartaceus, C. cuspidatus, C. giganteus, C. igneus, C. osae, C. spectabilis* and etc. The most commonly used species in the genus is *C. speciosus*, being an important antimicrobial plant, usually grown in moist, organic, fertile, well-drained soils away from direct sunlight. For its cultivation, tropical climate with minimum temperature of 13 ºC and high humidity is best (Eliza *et al.* 2008). *Costus* plant is about 1.5 m in length. It is a dramatic landscape plant with dark green large, subsessile, obovate or elliptic leaves arranged on the stalk in spiral way. It was cultivated in India crepe ginger which belongs to the family Costaceae (Zingiberaceae; Srivastava *et al.* 2011).

According to Srivastava *et al.* (2011), its classification is as follow:

Kingdom: plantae

Class: Liliopsida

Order: Zingiberales

Family: Costaceae

Genus: *Costus*

Species*: speciosus*

C. speciosus is traditionally used as a medicinal herb and considered as food and medicine (Ammal & Prasad 1984). It has diuretic, stimulant, digestive, carminative, and antiseptic properties. Juice of rhizome of the plant is applied to head for relief of headache, decoction of stem and bruised leaves are applied in fever, and in high fever, leaf infusion or decoction is used as sudorific or in a bath. Young stems and sap from leaves have many uses, e.g., against dysentery and diarrhoea, intestinal worms, vomiting, constipation, jaundice skin diseases, leprosy, cuts, wounds, scabies, antidote for snake bite, burning sensation, rash asthma, bronchitis, inflammations, nose pain, cough arthritis, anaemia, spermatorrhoea. It is also used as antivermin and for abortion (Sabitha *et al.* 2012). The rhizomes are usually used as decoction. An alkaloid extract from rhizomes had smooth muscle relaxant (papaverine like effect) and promotes antispasmodic activities. Rhizomes are also used in urinary diseases, jaundice, pneumonia, rheumatism, oedema, and leaves are used in mental disorders. The *C. speciosus* rhizome extract has non-estrogenic effect therefore stimulates the uterine contraction. The plant was used to treat eye and ear infections. Rhizomes have also cardiotonic, hydrocholeretic, diuretic and CNS depressant activities, and in Malaysia used for small pox. On eyelashes it is used to increase sexual attractiveness as an ingredient in cosmetics (Pawar & Pawar 2014).

Pseudomonas aeruginosa

In Greek, pseudo means false and (monad) means the unicellular microorganism. It is an obligate aerobic gram-negative bacterium, found single or in pairs or as short chain. It is 1.5-3 μm in length and its radius is 0.5-0.7 μm. It is also rod-shaped motile bacterium by means of a single polar flagellum, and does not ferment glucose (Brooks *et al.* 2010). The colour of cultures of the species in laboratory is blue green or verdigris which is the meaning of aeruginosa in Latin. This blue-green pigment results from a combination of two metabolites of *P. aeruginosa*, pyoverdine (green) and pyocyanin (blue; Brovon 1956). This bacterium can live and grow in environment with minimal nutrients and in water containing only traces of nutrients (tap water), so it can persist in the hospital environment. *P. aeruginosa* has a strong ability to resist disinfectants, which explains their role in hospital-

acquired infections. It is opportunistic pathogen, contains many virulent factors and functions as an important cause of the nosocomial infections. It is associated with high incidence of mortality and morbidity compared to other bacteria and has become a real cause of infection in immunocompromised patients or patients with burns after introducing a catheter or foreign material into the body.

MATERIALS AND METHODS

Preparations of media

In this study ready-made media, were prepared following the instructions of the manufacturing company. To ensure dissolution of all constituents completely, it was boiled in water bath, then sterilized in autoclave at 121 ^{o}C and 1.5 kg cm^{-2} for 15 min. The rest of culture media were prepared in the laboratory according to Nikhal *et al.* (2010).

Plant material

Costus speciosus rhizomes (Fig. 1) were obtained from herbalists' market in Baghdad City, Iraq and were identified by the Faculty of Agriculture, University of Baghdad. In order to prepare the sun-dried rhizomes for extraction, they were powdered using grinder.

Fig. 1. The rhizome of *Costus speciosus* plant.

Plant extraction

Alcoholic extractions

For alcoholic extractions, 250 g dried rhizomes powder were subjected to Soxhlet apparatus (Atlas *et al.* 1995) and extracted with 250 mL of 80% methanol in a ratio of 10:1 (Das *et al.* 2010). At first, we started the process by heating the extraction with methanol (solvent) in a beaker at a temperature of 40 ^{o}C. The coming up steam from the beaker was allowed to pass to the instillation unit through a tube connecting them together. The steam was then condensed due to passage of cold water in a spiral tube inside a glass cylinder. It started to come down as drops and entered the thimbles which contained the dry powder of the plant that was in the extraction unit, until the thimbles were filled with the solvent to which the plant compounds were transferred. When the extraction unit was filled, the solvent was left with what was melt from the plant material in the glass beaker by siphon process. By repeating the process and continuous evaporation of the solvent, the plant compounds were left in the beaker. As the process continued, the solvent inside the extraction unit became colourless and clear which was a sign to finish the extractions that lasted 24 hours. (Thakare 2004). The extract solution was filtered using Whatman No. 4 filter paper, then evaporated to remove the solvent by leaving it at room temperature for 2 days. The collected dried extract was frozen and stored until use for further experiments (Fig. 2).

Qualitative detection of active compounds in raw extract of *C. speciosus* rhizome

In order to detect the active compounds in the extract of the *C. speciosus* rhizome, a number of reagents were used. For each group of compounds, two detectors were used (Cannell 1998; Santhi *et al.* 2011; De *et al.* 2010).

Detection of alkaloids

A- we added few drops of Meyer reagent to 1 mL of the extract of the *C. speciosus* rhizome. A white precipitate indicates a positive result, which means alkaloids are present.

B- The same steps in the previous process were repeated using the Waknar detector, the presence of a brown precipitate indicates its existence.

Fig. 2. Step of alcoholic extraction of the plant rhizome; a & b: Both the methanolic and aqueous extracts after filtration; c & d: the dried extract after evaporation.

Detection of Saponites

A- Foam detector: 1 mL of *C. speciosus* rhizome extract is added to distilled water in a test tube. By tugging the tube strongly, the emergence of foam-dense indicates the presence of soap (Aritharan *et al.* 2012).

B - $HgCl_2$ detector: When a few drops of mercuric chloride were added to 1 mL alcohol extract of the *C. speciosus* rhizome, the emergence of white deposit indicates the presence of saponites.

Detection of terpenoids

A- Few drops of Anas aldehyde detector were added to 1 mL *C. speciosus* rhizome extract. The emergence of a brown precipitate indicates the presence of turbines.

B- By adding a mixture of concentrated sulfuric acid and chloroform to 1 mL alcohol extract, the appearance of reddish-brown precipitate indicates that turbines are present.

Detection of flavonoids

A- Concentrated sulfuric acid (H_2SO_4): One mL of the methanolic extract of the plant was added to few drops of concentrated sulfuric acid. Red colour of the resulting mixture means positive result.

B- Addition of crystals of magnesium (Mg): Adding drops of concentrated hydrochloric acid and crystals of magnesium to 1 mL of the alcoholic extract of the plant should reveal red colour, if the result is positive.

Detection of tannins

A- By adding few drops of lead acetate to 1 mL of the plant alcohol extract, the appearance of yellow deposit indicates the presence of tannins.

B- By adding drops of $FeCl_2$ to 1 mL of the alcohol extract, an orange deposit indicates the presence of tannins.

Aqueous extraction

Preparing aqueous extraction from roots and rhizomes of *C. speciosus*, according to Handa *et al.* (2008), is by mixing 25 g of the plant powder with 250 mL distilled water under boiling point in a proportion of 10:1, then leaving to cool with continuous shaking using the shaker. The solution is then filtered by layers of gauze and filtered again using filter papers (Whatman No. 2). Ultimately the leaky part was dried in the oven at 45-50 ºC till getting the dried powder.

The pathogenic bacteria

Pseudomonas aeruginosa was obtained from Department of Microbiology, College of Science for Women, University of Baghdad, Iraq. It was isolated from patients with urinary tract infection, and diagnosed through the Gram stain, biochemical test, and Vitek system (Table 1).

Table 1. The biochemical tests used to diagnose *p. aeruginosa.*

Test	*P. aeruginosa*
Gram stain	-
Motility	+
Oxidase test	+
Catalase test	+
H_2S production	-
Urease test	-
Citrate test	+

At first the bacterium was activated on brain heart infusion broth for 24 h at 37 ºC, then it was equalized with 0.5 McFarland standard (1.5×10^{-8}).

Experimental design

Alcoholic and aqueous extract concentrations were prepared by mixing 2 g of the extraction with 1 mL DEMSO followed by adding 7 mL sterile distilled water, so we had the first concentration of 200%. Then 9 mL sterile distilled water were added in 4 tubes, in order to obtain serial dilution with concentrations of 100%, 50% and 25% (Fig. 3).

Antimicrobial assay

The methanolic and aqueous extract of the plant were tested against *P. aeruginosa*. To screen the antimicrobial activity, the well diffusion method was used (Saraf 2010). A plate of Mueller Hinton agar was streaked with 200 µL *P. aeruginosa*, then a well of 6 mm diameter on the surface of the inoculated agar plate was filled with 40 µL of each extract solution with a certain concentration (Fig. 4). The compound was left and allowed to diffuse for 5 minutes, then the plates were incubated at 37 °C for 24 h. When the incubation period reached the end, inhibition zones around the well were measured in mm using transparent ruler. The study was performed in triplicate. A well filled with distilled water was used as a negative control.

Fig. 3. Serial dilutions of alcoholic extraction by concentrations of 200%, 100%, 50% and 25% with control.

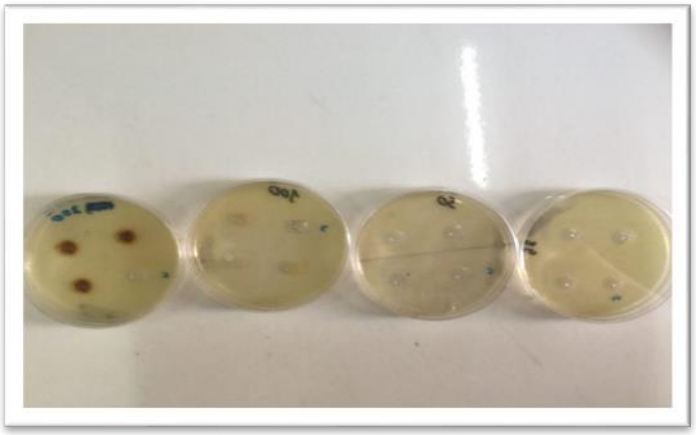

Fig. 4. The Mueller Hinton agar plate streaked with *Pseudomonas aeruginosa* and the well filled with plant extract.

RESULTS AND DISCUSSION

Qualitative detection

The results of qualitative detection of the active compounds (Table 2) showed the existence of five compounds in the methanolic extract of *C. speciosus* rhizomes. These compounds were alkaloids, saponites, turbines, tannins, and flavonoids. The presence of these active compounds in the extract is very important due to exhibiting antimicrobial activity. Chemical substances that produce clear physiological actions on human bodies, are accumulated in the storage organs of the plants, so these organs reveal very important biological activities in higher plants. Of these bioactive compounds the most important are alkaloids, flavonoids and phenolic compounds (Buwa & Staden 2006).

The antibacterial activity assay

According to the results obtained, the maximum antibacterial activity of the methanol and aqueous extract of *C. speciosus* was with a concentration of 200 µg mL^{-1} and an inhibition zone diameters of approximately 16 and 17

mm respectively (Figs. 5a and b). The methanolic extract was moderately effective with a concentration of 100 µg mL^{-1} and an inhibition zone diameter of approximately 10 mm (Fig. 6), while no inhibition was obtained on the tested bacterium with the other concentrations.

Table 2. Specific detection of some active compounds of the alcoholic extract of *C. speciosus* rhizome.

Active Materials	Reagent A	Reagent B
Alkaloids	+	+
Saponites	+	+
Turbines	+	+
Tannins	+	+
Flavonoids	+	+

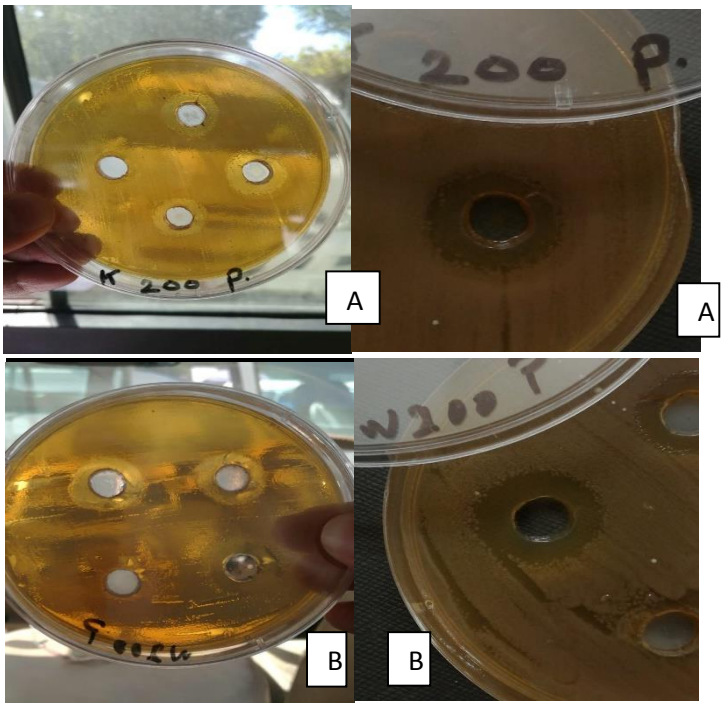

Fig. 5a. The methanolic extract with a concentration of 200 µg mL^{-1} and an inhibition zone diameter of approximately 16 mm; **b:** The aqueous extract with a concentration of 200 µg mL^{-1} and an inhibition zone diameter of approximately 17 mm.

Antibacterial activity of the plant was studied by several authors (Joji & Benna 2010; Aritharan *et al.* 2012; Manorama *et al.* 2013), reporting that there was high antibacterial activity of rhizome extract against both Gram-positive (*Staphylococcus aureus, S. epidermidis*) and Gram-negative bacteria (*Escherichia coli, P. aeruginosa, Salmonella typhimurium*). This explains the plant beneficial effects on diseases, since the essential oil derived from the rhizomes contains many active compounds such as a precursor for the synthesis of steroidal hormones called diosgenin, in addition to phenolics and alkaloid substances (Ariharan *et al.* 2012) as well as steroid saponins and sapogenins (Sabitha *et al.* 2012).

Fig. 6. The methanolic extract was moderately effective at 100 µg mL^{-1} with an inhibition zone diameters of approximately 10 mm.

Also, Nehetea *et al.* (2010) declared that several properties of plant products are attributed to the presence of phenolic compounds, which are necessary for plant development and improve their defence mechanisms. The addition of these compounds in the regular diet might lower the incidence of diseases and be beneficial to human health (Nehetea *et al.* 2010).

CONCLUSION

According to this *in vitro* study, it was found that the methanolic extract of *C. speciosus* rhizomes as an inhibitory factor exhibited a significant effect on the growth rate of *P. aeruginosa* with an optimal concentration of 200 mg mL^{-1}. The aqueous extract of *C. speciosus* rhizomes as an inhibitory factor displayed a significant effect on the growth rate of *P. aeruginosa* with an optimal concentration of 200 mg mL^{-1}. There was a negative correlation between the growth rate of *P. aeruginosa* and the concentration of *C. speciosus*

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