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A study of Anti-bacterial, Anti-fungal Activities of Ethanolic and Aqueous Extracts of *Costus speciosus*

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Abstract The *Costus speciosus* belonging to family Coastaceae, is a substantial medicinal and ornamental plant used traditionally to cure different diseases. *Aim:* The current studies were sought to determine the antimicrobial activities of ethanolic and aqueous extracts of *Costus speciosus* rhizome (CSEE and CSAE) against nine bacterial strains (*P. aeruginosa, S. epidermidis, S. pygenes, E. coli, K. pneumonia , S. Typhimurium, Proteus mirabillus, Bacillus species*) and three fungal strains (*Aspergillus fumigates, Penicillum species and Fusarium species*). *Method:* The ethanolic and aqueous extracts were obtained by hot and cold maceration methods and the antimicrobial effect was found using well diffusion method. *Results:* All the nine tested bacteria and three tested fungi showed concentrations dependent susceptibility to both ethanolic extracts (CSEE). The aqueous extract on hot has antibacterial activity against *P. aeruginosa, and S. aureus* only and has no antifungal activity. The highest antibacterial activity was exhibited by both extracts (CSEE and CSAE) against *P. aeruginosa* (24mm). *K. pneumonia* and *E. coli* were susceptible to the highest concentration of on hot and on cold CSEE, while, *S. Typhi* was affected by CSEE on cold and *proteus mirabilis* was susceptible to on hot CSEE. Additionally strong antifungal activity was exhibited by CSEE against *Penicillum species, Fusarium species and Aspergillus fumigatus,* the highest inhibition zones were 37mm, 23mm and 21mm respectively. Whilst, the CSAE was not elucidate any activity against fungal strains. Overwhelmingly, the antimicrobial activities were due to biological constituents that present in *C. speciosus* rhizomes such as steroidal saponins (diosgenin), sesquiterpenoid compounds (costunolide and eremanthin), alkaloids, and other chemical constituents. *Conclusion:* On the basis of these finding, it may be inferred that ethanolic extracts of *C. speciosus* rhizomes on hot and on cold in high concentrations have a good antibacterial and antifungal activities against various pathogenic microorganisms. Whilst, aqueous extract on hot has antibacterial activity against *P. aeruginosa, and S. aureus* only and has no antifungal activity.

Keywords *Costus speciosus, Pseudomonas aeruginosa, Aspergillus fumigates, Penicillum species*

Introduction

Costus speciosus (Linn) is an Indian ornamental and important medicinal plant, belonging to family Coastaceae (Zingiberaceae) [1, 2] which is often called spiral ginger. Family Zingiberaceae is a family of about 52 genera and more than 1,300 species [3]. The genus Costus comprises 175 species like: *Costus afer*, *Costus arabicus*, *Costus speciosus*, etc. C. speciosus also known as keukand, keu and grepe ginger is widely distributed throughout the world [4, 5], it occurs in the humid tropics area of the Indo-Malayan region, Sri Lanka, hills of India and Himalayas [3].

The plant is a succulent, upstanding, perennial, ornamental, herbaceous, tuberous stem, sub-woody at the base, thick creeping rhizomes [6] growing up to 2-2.7 m height with long lanceolate leaves and white fragrant flowers in terminal clusters [4,7]. The rhizomes have brownish colure with incense odor. The rhizome and aerial parts of the plant are edible.

The phytochemical analysis of *C. speciosus* rhizome has revealed its richness in carbohydrate, starch, amylase, protein, lipid and vitamin A [8]. Moreover, the rhizomes are wealthy in bioactive substances as quercetin, rutin, luteolin, kaemphrol and coumarin [9]. Also it contains antioxicant components like β- carotene, Vitamin C, Vitamin E and traces elements as nitrogen, calcium, potassium, sodium and magnesium [10]. Karthikeyan *et al.*, 2012 reported that *C. speciosus* contains proteins and phenolic compounds [11]. Other researchers exhibited that the rhizomes are a good source of saponin like diosgenin [12], prosapogenin B of dioscin, cycloartenol, 25 cycloartenol, and steroidal saponin [13, 14]. Additionally, it contains steroids and alkaloids [15, 16], triterpenes, and corticosteroids [17]. These phytoconstituents of *C. speciosus* rhizomes have been shown to exert beneficial biological and pharmacological effects including the anticholinesterase activity [18], antihelmentic [19], larvicidal [15], antioxidant [20], anti-inflammatory, analgesic and antipyretic activities [21]. Additionally different extracts of *C. speciosus* rhizomes have been shown antihyperglycemic effect [17], antistress [22], cardiotonic and diuretic effect [16]. The plant has also been reported to possess cytotoxic antitumor activity, anti-fertility, hepatoprotective activity [7] and antibacterial and antifungal activity [23, 24].

The present study interested to investigate *In vitro* effects of hot and cold ethanolic (CSEE) and aqueous (CSAE) extracts of *Costus speciosus* (CP) against nine pathogenic bacterial strains (*Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus pygenes, Escherichia coli, Klebsella pneumonia , Salmonella Typhimurium, Proteus mirabillus, Bacillus species*) and three pathogenic fungal strains (*Aspergillus fumigates, Penicillum species and Fusarium species*).

Materials and Methods

Plant material

The rhizome of *C. speciosus* was obtained from herbal pharmacy in Misurata, Libya and was identified by the Dr. Elsidieq Ali Kshim, specialist in agriculture. The roots were washed with tap water, dried and ground well. The fine powder obtained was extracted by ethanol and water on hot and cold methods. All extracts were tested for their antibacterial and antifungal activities.

Preparation of plant extracts

The shade dried rhizomes were powdered with grinder and then passed via sieve with 40 meshes. Exactly 150 gm of dried rhizomes powder subjected to extraction on hot and cold using ethanol (99.8%) and distilled water as solvents. All the dried extracts were kept at a low temperature (4 - 8 °C) in air tight containers for further uses.

Ethanolic extraction (on cold)

Fifty grams of the rhizomes powder were macerated into 100 ml of ethanol (1:2 w/v) for 48 h at room temperature away from the light, with continues shake using science lab orbital, then the mixture was filtered twice time through Whatman No. 4 filter paper. The filtrate was concentrated in a rotary evaporator in a vacuum at 60 ºC and dried further at 45 °C. The extract was stored until used for experiments.

Ethanolic and Aqueous extraction (on hot)

The *C. speciosus* rhizomes were prepared as explained in previous section. For ethanolic extraction, 50 grams of the powder was placed in flask and macerated into 100 ml of ethanol for 48 h. For aqueous extraction, 50 grams of the rhizome powder was macerated in flask into 100 ml of sterile distilled water at room temperature for 48 h. Then the contents of both flasks were putted separately in rotary evaporator for 24 h at 70 ºC. The mixtures were filtered twice time via Whatman No. 4 filter paper. Samples were poured in rotary evaporator to remove as much as possible extra ethanol and water. Then the concentrated samples were dried further using oven at 45°C. The dried samples were stored until used.

Microorganisms and media

Nine pathogenic bacterial strains (*Pseudomonas aeruginosa, Staphylococcus epidermidis, Streptococcus pygenes, Escherichia coli, Klebsella pneumonia, Salmonella Typhimurium, Proteus mirabillus, Bacillus species*) and three pathogenic fungal strains (*Aspergillus fumigates, Penicillum species and Fusarium species*) were used in this study. All microorganisms were purchased from Food and Drug Control Center, Misurata, Libya, and identified according to standard phenotype tests. Mueller- Hintorn Agar medium (MHA) and Sabauraud Dextrose Agar (SDA) medium were used for cultivation of the pathogenic bacteria and fungi respectively at 37 °C. All the media were autoclaved before culturing.

Antimicrobial activity of *C. speciosus* rhizome extracts

Antibacterial and antifungal activities of hot and cold ethanolic and aqueous extracts of *C. speciosus* (CSEE) and (CSAE) were evaluated using Agar Well diffusion method on MHA and SDA respectively against above nominated microorganisms. *P. aeruginosa* was used as a reference for the antibacterial assay while *Aspergillus fumigates* was used as a reference for the antifungal assay of the extracts. Twenty four hours few pure colonies on broth were used to prepare the bacterial and fungal suspension for each strain, with the turbidity of 0.5 McFarland. The extracts were dissolved in sterile distilled water. The initial concentration of each extract was 500 mg/ml as stock solution. The initial test concentration was serially diluted four-fold. The MHA and SDA plates were prepared by pouring 15 ml of media into sterile Petri dishes and allowed to solidify for 5 minutes. Six wells were punched in each plate using sterile harden gimlet (6mm diameter). MHA and SDA agar plates were inoculated uniformly with bacterial and fungal strains suspension using sterile cotton swabs under aseptic conditions and allowed to dry for 5 minutes. Then the wells were filled with 100 µl of different concentrations (1:1, 1:2, 1:4, 1:8, 1:16) of each extract solution and allowed to diffuse for 10 minutes. The plates were incubated at 37°C for 24 h for bacteria and 72 h for fungal plates. At the end of incubation, inhibition zones formed around the wells were measured with transparent ruler in millimeter (mm). Table 1 represents concentrations of the samples.

Table 1: Dose of the extract in each concentration

Dilution	1:1	1:2	1:4	1:8	1:16
Dose (mg/ml)	500	250	125	62.5	31.25

Results

In the present investigation, extraction of *C. speciosus* rhizome with ethanol on hot and cold was carried out. All the nine tested bacteria and three tested fungi showed susceptibility to both ethanolic extracts (CSEE). The Gram negative bacteria *Pseudomonas aeruginosa* was susceptible to all hot ethanolic concentration and the maximum inhibition zone was 24mm at 1:1 conc. while, the minimum one was 12mm at 1:16 conc. while, the on cold CSEE induced 19 and 8 mm zones inhibition at the two highest concentrations. The other Gram negative bacteria *S. typhi* was susceptible only to on cold CSEE, while *Proteus mirabilis* was susceptible to on hot CSEE. Also, *E. coli* was susceptible to high concentrations of on hot and on cold CSEE. Additionally, all the Gram positive bacteria (*Bacillus spp*.**,** *S. epidermidi*s, *S. aureus*, *Strept.* group A) in this study were susceptible to high concentration of both C. speciosus ethanolic extracts (Table 2). Out of the nine pathogenic bacterium and three fungal stains, only *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found to be the susceptible organisms to hot aqueous *C. speciosus* rhizome extract (CSAE). All preparations of CSAE exhibit concentration dependent antibacterial activity against *Pseudomonas aeruginosa.* The maximum zone inhibition was 24 mm at 1:1 conc. while, the minimum zone inhibition was 10mm at lowest 1:16 conc. Also *S. aureus* zones inhibition were 10 and 8 mm at 1:1 and 1:2 conc. respectively (Table 2).

Results of antifungal activity of on hot and on cold *C. speciosus* ethanolic extract are summarized in Table 3 and Figure 1a & 1b. The extracts revealed that all the tested fungus exhibited susceptibility to CSEE. Most CSEE concentrations on cold showed concentration dependent zones inhibition of fungus growth. The concentration 1:1 showed 37mm, 23mm, 21mm zones inhibition against *Penicillum sp*, *Fusariam sp.*, and *A. fumigatus* respectively. On the other hand CSEE on hot showed less activity against tested fungus than CSEE on cold. The minimum

inhibitory concentration of *Penicillum sp. and Fusariam sp.* was at 1:2. Unfortunately, all the three fungus stains were resistant to on hot as well as on cold CSAE.

Table 2: Antibacterial activity of *Costus speciosus* rhizome extracts

Bacteria	**CSEE**										**CSAE**				
	On hot					**On cold**					**On hot**				
	Concentration of extracts and inhibition zone in mm														
	1:1	**1:2**	**1:4**	**1:8**	**1:16**	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**
Gram negative															
p. aeruginosa	24	22	19	14	12	19	8	-	-	-	24	18	15	13	10
K. pneumonia	21	13	-	-	-	21	13	-	-	-	-	-	-	-	-
*S. Typhi***.**	-	-	-	-	-	17	10	-	-	-	-	-	-	-	-
proteus mirabilis	11	10	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	9	-	-	-	-	16	6	-	-	-	-	-	-	-	-
Gram positive															
*Bacillus spp***.**	12	10	-	-	-	26	18	10	-	-	-	-	-	-	-
S. epidermidis	9	-	-	-	-	15	7	-	-	-	-	-	-	-	-
S. aureus	12	-	-	-	-	10	8	-	-	-	10	8	-	-	-
Strept. group A	9	9	-	-	-	15	-	-	-	-	-	-	-	-	-

CSEE= *Costus speciosus* ethanolic extract, CSAE= *Costus speciosus* aqueous extract,
S. aureus = Staphylococcus aureus, S. epidermidis = Staphylococus epidermidis, Bacillus Spp. = Bacillus species, Strept. group A= *Streptococcus pyogenes. P. aeruginosa= Pseudomonas aeruginosa, E. coli = Escherichia coli, S. Typhi. = Salmonella Typhimurium, K. pneumonia= Klebsiella peneumonia*.

Table 3: Antifungal activity of *Costus speciosus* rhizome extracts

Fungus	**CSEE**										**CSAE**				
	On hot					**On cold**					**On hot**				
	Concentration of extracts and inhibition zone in mm														
	1:1	**1:2**	**1:4**	**1:8**	**1:16**	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**
Penicillum spp	13	11	-	-	-	37	17	10	9	-	-	-	-	-	-
Fusariam sp.	16	9	-	-	-	23	12	8	6	-	-	-	-	-	-
A. fumigatus	17	12	8	-	-	21	20	7	-	-	-	-	-	-	-

CSEE= *Costus speciosus* ethanolic extract, CSAE= *Costus speciosus* aqueous extract

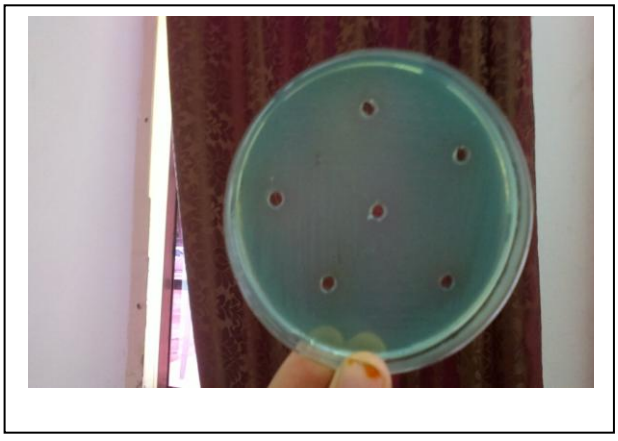

Figure 1a: Asperogillus fumigatus (control)

Figure 1b: Effect of on cold CSEE on Asperogillus fumigatus

Discussion

Infectious diseases are common worldwide and presumed causes of morbidity and mortality in the world. Furthermore, the disaster occurs when different species of pathogenic microorganisms continuously developed resistance to clinically used antibiotics. Additionally, serious adverse effects, and low efficacy of certain antibiotics induce significant hindrance for both the physicians and the patients. Historically, most of the medicinal preparations were derived from plants. Nowadays there are a lot of effective drugs that are developed from plants [26]. According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [27]. These concerns have led to explore the nature for novel antibiotics that could be used to combat microbial infections. The current study aimed to evaluate the antimicrobial activity of CSEE and CSAE against nine pathogenic bacterium and three pathogenic fungi. Our findings indicate that five Gram negative and four Gram positive tested pathogenic bacterium are susceptible to different concentrations of both on hot and on cold ethanolic and only on hot aqueous *C. speciosus* rhizome extracts. Moreover, the three tested pathogenic fungi are sensitive to on hot and on cold CSEE only. Among the tested bacteria, *Pseudomonas aeruginosa* was found to be the most susceptible studied bacterium, whereas it was sensible to all on hot concentrations (CSEE and CSAE) ranging from (1:1 – 1:16), the maximum inhibition zones were 24mm. Whilst, the other tested microorganisms as well as *P. aeruginosa* were un susceptible to on cold CSAE. Seemingly, the high temperature activates the constituents of *C. speciosus* aqueous extract that possess antimicrobial activity Table-3. Tiwari *et al*., 2011 indicated that, the traditional method of treating a bacterial infection was by administering a decoction of the plant or apart there by boiling it in water [28].

Our results are in line with the study of Shaikh Fakhra *et al.,* 2014, who reported that the on hot, on cold and its silver nanoparticles of *Costus speciosus* leaves aqueous extracts were effective against *P. aeruginosa, E. coli, K. pneumonia, Enterobacter* sp., *and Proteus mirabils.* Also, they found that out of the five pathogenic isolates, *Pseudomonas aeruginosa* was found to be the most susceptible organism [29]. On the other hand, Ariharan et al., 2012, reported that aqueous C. speciosus rhizome extract possess good antibacterial activity against pathogenic strains of Gram negative (*E. coli, P. aeruginosa and S. typhimurium*) and Gram positive (*S. aureus, S. epidermidis*) as compared to standard drug gentamycin [6]. *E. coli* occurs as a commensal in the gut of human and animals and its presence in water supplies is regarded as an index of faecal contamination. *E. coli* is frequently involved in infections of the urinary tract; cystitis and pyelonephritis. *E. coli* may cause gastro-enteritis of infants, and is a common cause of neonatal septicaemia and meningitis. *E. coli* may be involved in abdominal or pelvic infections. Post-operative complications such as stitch abscess, wound sepsis or peritonitis may caused by *E. coli*. The organism is typically penicillin-resistant and is often resistant to various other antibiotics. In our study *E. coli* was susceptible only to high concentration of CSEE, the maximum inhibition zone was 16mm.

In the present study both extracts of *C. speciosus* on hot and on cold CSEE and on hot CSAE induced zones inhibition of *S. aureus* at high concentration (1:1, 1:2) Table-2. Sulakshana et al., 2013, reported that the petroleum ether extract of *C. speciosus* rhizomes exhibited antimicrobial activity against *S. aureus, Bacillus subtilis, E. coli, and P. aeruginosa* when used at different concentrations (500-2000 µg) [30]. The Gram positive bacteria such as *Staphylococcus,* particularly *Staphylococcus aureus* has been recognized as an important cause of systemic and superficial infections such as post-operative wound infections, pneumonia, bacteremia, and infections of the bone, wounds and food poisoning. *S. aureus* can also produce superficial infections such as boils, impetigo and folliculitis [31]. Moreover, *S. aureus* is uniquely equipped with virulence factors and defense mechanisms that could cause rapidly progressive fatal infection [32, 33]. Malabadi (2005), reported that hexane and methanol extracts of leaf and rhizomes of *C. speciosus* possess antibacterial activity against various pathogenic strains isolated (*Shigella, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas, Bacillus subtilis* and *Saltmonella*) from infected patients, whilst, the aqueous extract has no bacterial activity against these strains [34]. Whilst, Saraf *et al.,* 2010, reported that the aqueous and methanol extracts of *C. specious* rhizome did not exhibit any antibacterial effect against *E.coli, S. aureus, K. pneumonia* and *P. aeruginsa* [35]. The ethanol extract on hot and on cold of *C. specious* rhizomes (CSEE) exhibited inhibitory activity against three studied fungal strains (*Penicillum species, Fusarium species and Aspergillus fumigates*). These inhibitions were occurred at the

concentrations ranging from (1:1 – 1:4) in case of on hot extract and ranging from (1:1 – 1:8) in case of on cold extract. However, the sensitivity varied among them. High inhibition zones were observed in *Penicillum spp* (37 mm) followed by *Fusariam sp*. (23 mm). *A. fumigates* showed low inhibition zone (21 mm). Unfortunately, all the three fungal strains were resistant to both CSAE. The study conducted by AL-Ameri *et al.,* 2013, has shown that the methanolic rhizome extract of *C. speciosus* possess antifungal activity in vivo, where it inhibit *Aspergillus fumigates* that induce pneumonia in rats [36]. Other researchers were reported that the hexane extract of *C. speciosus* rhizomes have good activity against *Trichophyton mentagrophytes, T. simii, T. rubrum , Epidermophyton floccosum, Scopulariopsis* sp, *Aspergillus niger, Curvulari lunata*, and *Magnaporthe grisea*. The researchers attributed this activity to presence of two sesquiterpenoid compounds (costunolide and eremanthin) particularly, costunolide, but these two constituens have no activity against the tested bacteria (*S. aureus*, *S. epidermidis* and *Bacillus subtilis)* [37]. Also, its rhizome extract has activity against *Candida albicans* [38]. Edeoga *et al.,* 2005, said that the "The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body" [39]. *C. speciosus* rhizomes contains various bioactive constituents such as steroidal saponins (diosegenin), prosapogenin B of dioscin, sesquiterpenoid compounds (costunolide and eremanthin), alkaloids, triterpenes, corticosteroids, flavanoids and other effective compounds that have antibacterial and antifungal activities.

Conclusion

On the basis of these finding, it may be inferred that ethanolic extracts of *C. speciosus* rhizomes on hot and on cold in high concentrations have a good antibacterial and antifungal activities against various pathogenic microorganisms. Whilst, aqueous extract on hot has antibacterial activity against *P. aeruginosa, and S. aureus* only and has no antifungal activity. Unfortunately, no antibacterial effect was recorded with aqueous extract on cold against all the tested microorganisms. These activities were related to the presence of different bioactive constituents.

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Conflict of Interest

The authors humbly declare no conflicts of interest regarding publishing this research.

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