

Full Length Research Paper

Latent natural product and their potential application as anti-infective agents

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Extract of *Acorus calamus* (AC) and *Ferula asafetida* (FA) were tested against different bacterial pathogens by well-cut agar diffusion method. To analyzed the *in vitro* activities of *A. calamus* and *F. asafetida*, four different bacteria (*Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*) were used. *A. calamus* had a broad spectrum antimicrobial effect against different bacterial pathogens. Streptomycin was used as standard drug with significant activity values, that is, 34 mm against *S. aureus*, 36 mm against *S. epidermidis*, 30 mm against *B. subtilis* and 24 mm against *E. coli*. Analysis of the data showing that, the crude extract of *A. calamus* in n-hexane exhibited superior activity against *S. epidermidis*. The dichloromethane extract of *F. asafetida* was found low against *E. coli* and *B. subtilis*. Results were compared concomitantly to standard drugs: streptomycin. Phytochemical screening of *A. calamus* and *F. asafetida* showed the presence of terpenoids, saponins, flavonoids, alkaloids, tannins, glycosides and reducing sugar components. The high potency of *A. calamus* and *F. asafetida* against these microbes could provide an example of prospecting for new compounds. Based on the current conclusion, it can be accomplished that these plants have antimicrobial activity, which is as potent as standard antimicrobial drugs against specific microorganisms.

Key words: *Acorus calamus*, *Ferula asafetida*, infection, inhibition, phytochemical screening, medicinal plants.

INTRODUCTION

The phytochemical research, in which ethno pharmacological properties of plants are evaluated, leads to the discovery of new anti-infective agents from higher plants. Owing to the development of drug resistive strains in human pathogens against commonly used antibiotics, it is necessary to discover new antimicrobial substances from plants and other sources. Popular European books on medicinal plants touted *calamus* as a "wonder drug." It was commonly used in folk medicine as a "nervine," most likely linked to the tranquilizing effect of cis-isoasarone. In Exodus 30: 23, 24, 34, it has been written that God ordered Moses to make the Holy Oil, one of its constituents was an aromatic reed which some authorities have suggested might have been *Acorus calamus*. Some

people strung together its dried root pieces, steam it throughout the home and is thought to "kill" sickness. *A. calamus* Linn, commonly known as sweet flag, is an aromatic medicinal plant belonging to the Araceae family. It has been long known for its medicinal value, it is wild or cultivated throughout the Himalayas at an altitude of 6000 ft. The rhizomes of *A. calamus* contain an aromatic oil that has been used medicinally since ancient times and has been harvested commercially (Meena et al., 2010). The most common way of ingesting *A. calamus* is by chewing it. It can be peeled and washed to remove the bitterness and then eaten raw like a fruit. The dried and powdered rhizome has a spicy flavor and is used as a substitute for ginger, cinnamon and nutmeg. A pinch of

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the powdered rhizome is used as a flavoring in tea. The inner portion of young stems makes a very palatable salad. Sweet flag has a very long history of medicinal use in Chinese and Indo-Pak herbal traditions (Khan, 2012).

It is widely employed in modern herbal medicine as its sedative, laxative, diuretic, and carminative properties (Marcy et al., 2005). It is used in Ayurveda to counter the side effects of all hallucinogens. Both roots and leaves of *A. calamus* have shown strong antioxidant (Devi et al., 2011). It is used as antimicrobial against various bacteria, filamentous fungi, and yeast (Balakumbahan et al., 2010). *A. calamus* is effective against cattle tick, *Rhipicephalus (Boophilus) microplus* (Gosh et al., 2011). A recent study showed that beta-asarone isolated from *Acorus calamus* oil inhibits adipogenesis in 3T3-L1 cells and thus reduces lipid accumulation in fat cells (Johnson et al., 1995). Protective effect against acrylamide induced neurotoxicity (Lee, 2011). Chewing the root of *calamus* helps fight tobacco addiction, that is, it kills the taste for tobacco over time (Shukla et al., 2006). The plant is externally used to treat skin eruptions, rheumatic pains and neuralgia. It is used in incense sticks and is widely used as insecticide for lice, bedbugs, worms, etc. Sweet Flag is used as an antihistamine (Brown et al., 1989). *A. calamus* uses are abortifacient, anodyne, aphrodisiac, aromatic, carminative, diaphoretic, emmenagogue, febrifuge, hallucinogenic, homeopathy, odontalgic, sedative, stimulant, stomachic, tonic, and vermifuge.

Ferula is a genus of about 170 species of flowering plants in the family Apiaceae. It is native to the Mediterranean region East to Central Asia, mostly growing in arid climates. It is strictly distributed in Pakistan, India, Iran, and Afghanistan. *Ferula asafetida* has a pungent, unpleasant smell when raw, but in cooked dishes, it delivers a smooth flavor, reminiscent of leeks. Plant prefers full sun and dry soil (Morningstar and Desai, 1991). *F. asafetida* has shown remarkable antioxidant and antihemolytic activities. It reduces the growth of indigenous microflora in the gut, reducing flatulence. It is effective against Swine flu virus. Used for treating chronic bronchitis and whooping cough, as well as reducing flatulence.

F. assafoetida has also been reported to have contraceptive/abortifacient activity, *F. assafoetida* oleo-gum-resin has been reported to be antiepileptic. Roots yield an oleo-gum-resin used as an expectorant, antispasmodic and spice inhaling this gum prevents hysterical attacks. *F. asafetida* is useful in alleviating toothache. This spice is used as a digestive aid, in food as a condiment and in pickles (Wichtl, 2004).

MATERIALS AND METHODS

Plant

Rhizome of *A. calamus* and gum of *F. asafetida* were collected from Malakand, Pakistan. The taxonomic identification of both plants was carried out by Dr. Farrukh Hussain at the Department of Botany

(DOB), University of Peshawar, Pakistan. A voucher specimen under the scientific name of the plant (bot. 20012 (pup) and bot. 20013 (pup)) was deposited at the herbarium of Department of Botany, University of Peshawar, Pakistan.

Preparation of crude extract

Plant materials were dried under shade and powdered. From these powdered materials, weighed amount was taken in separate thimbles. This was suspended above the flask containing the solvent n-Hexane fitted with reflux condenser. The flask was heated (60 to 65°C for 5 to 6 h) on heating mantle; the evaporated solvent upon condensation trickled into the extraction chamber containing the plant material. At the end of the extraction process, the flask containing the n-Hexane extract was removed. After the removal of n-Hexane extract, the plant material remains in thimble and the next solvent of high polarity was passed like dichloromethane and extract was obtained. Ethyl acetate at the end of highly polar solvent ethanol was passed and thus different extract were obtained according to increasing polarities of solvents. The solvents obtained were evaporated under vacuum using rotary evaporator. The plants extracts were collected in labeled vials. The plant materials taken for extraction were *A. calamus* (70 g) and *F. assafoetida* (65 g). The crude extracts were subjected for antibacterial activity and their phytochemical screening.

Qualitative analysis of chemical constituents

Chemical analysis for the presence of major classes of secondary metabolites (alkaloids, tannins, anthraquinones, glycosides, reducing sugars, saponins, flavonoids, phlobatanins, steroids and terpenoids) in the crude extracts was carried out according to the method described by Pearson (1976).

Antimicrobial assay

Preparation of medium

Two types of media were used, solid and liquid media. Nutrient agar 16.8 g was dissolved in distilled water and volume was made up to 0.6 L. It was autoclaved at 120°C for 15 min. Media was cooled and allowed to solidify in Petri dishes. Nutrient broth 1.9 g was dissolved in distilled water and volume was made up to 150 ml. 8 ml of this broth was added to screw capped test tubes, which were placed in autoclave at 120°C for 15 min, then refrigerated at 37°C.

Preparation of tested materials

The test sample were prepared by dissolving 21 mg of crude extract in 1 ml of DMSO in Eppendorff tube and kept for 1 h. To another Eppendorff tube, 1 mg of streptomycin was dissolved in 1 ml DMSO and kept for 1 h.

Antibacterial assay

Cup-plate diffusion method

Antibacterial activity of plant extracts was carried using cup-plate agar diffusion method (14) with some small modifications. One milliliter from each standard bacterial stock suspension was mixed thoroughly with 40 to 45 ml of sterile Molten Mueller-Hinton agar 40°C, poured into sterile Petri-dishes and left to solidify. Then, four

cup-shape wells (120 mm diameter) were made in each plate using sterile cork-borer. The agar disks were removed and four alternate cups were filled with extract using sterile adjustable pipettes. Four Petri-dishes with two alternate cups were used with the respective solvent instead of the extracts as control. The plates were then incubated in upright position for 22 to 24 h at room temperature. Two replicates were carried out for each extract. After incubation period, the inhibition zones diameters were measured.

Disc diffusion method

The antibacterial assay for plant extract was also conducted using disc diffusion method as illustrated by Abdel-Wahab et al. (2009). The nutrient agar solution (16 ml) was poured and kept overnight in a refrigerator. Whatman filter paper discs of 6 mm diameter were impregnated with 10 µl of the solution of crude extract (at 4 mg/ml) dissolved in dimethyl sulfoxide (DMSO). Standard disc of streptomycin sulphate (10 µg/disc) was used as positive control, while DMSO was used as a negative control. The Petri dishes were inverted and incubated for 24 h at 37°C. Clear inhibition zones around the discs indicated the presence of antimicrobial activity.

RESULTS

Phytochemical screening

Phytochemical screening of chemical constituents of crude extract in different solvents showed the presence of alkaloids, tannins, anthraquinones, glycosides, reducing sugars, saponins, flavonoids, phlobatanins, steroids and terpenoids constituents as shown in Tables 1 and 2, according to Syed et al. (2013).

Antimicrobial screening

The results were summarized in Table 3. However, results were interpreted in terms of commonly used terms: sensitive, intermediate and resistant. Findings of cup-plate diffusion method for ethanolic extracts of *A. calamus* and *F. asafetida* are exposed as shown in Figures 1 and 2.

Antibacterial activities of *A. calamus* and *F. asafetida*

The antibacterial activity of crude extracts, that is, n-Hexane, dichloromethane, ethyl acetate and ethanol of the selected plants. Traditionally, *A. calamus* and *F. asafetida* were used to treat bacterial infections. The results from the current study were screened for their microbial activity against four standard bacteria, namely, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Escherichia coli*. The pattern of inhibition varied with the plant's extract, the solvent used for extraction and the organism tested. DMSO was used as negative control and no inhibition was shown by it against all the bacteria. Streptomycin, an antibiotic was used as a positive control. Streptomycin was used as

standard drug with significant activity values (Figure 3).

Results of the antibacterial activities of the plants extracts are summarized in Table 3. For all the crude extracts, the highest antibacterial activities were shown by n-hexane and ethyl acetate crude extract of *A. calamus* followed by dichloromethane fraction of *F. asafetida*. The n-hexane extract of *A. calamus* showed good activity against *S. epidermidis*. Non-significant activity of n-hexane extract of *A. calamus* was seen against *E. coli* and *B. subtilis*, while no activity was shown against *S. aureus*. Non-significant activity was seen for dichloromethane extract against *E. coli* and *B. subtilis*, while no activity was seen against *S. aureus* and *S. epidermidis*. The ethyl acetate of *A. calamus* showed low activity against *S. epidermidis*, non significant against *B. subtilis* and *S. aureus*, while no activity was seen against *E. coli*. *A. calamus*, ethanolic extract showed no activity against *B. subtilis*, *S. aureus* and *E. coli*, while non significant against *S. epidermidis*.

The dichloromethane extract of *F. asafetida* was found low against *E. coli* and *B. subtilis*. While exhibited non-significant activity against *S. epidermidis* and no activity against *S. aureus*. The n-Hexane extract of *F. asafetida* showed no activity against *S. epidermidis* and *E. coli* while non-significant against *S. aureus* and *B. subtilis*. The ethyl acetate extract was found non-significant against *B. subtilis*, *S. aureus* and *E. coli*, while no activity was seen against *S. epidermidis*. In addition, *F. asafetida* ethanolic extract showed non-significant activity against *S. epidermidis* and *E. coli* and *B. subtilis* while no activity was seen against *S. aureus*.

DISCUSSION

In the present study, the n-Hexane extract of *A. calamus* (2 mg/ml) showed that it is potent against bacterial strains as compared to its other extracts as shown in Figure 1. The zone of inhibition was 16 mm against *S. epidermidis*. So this plant can be further processed for isolation of active components, inhibiting the growth of this pathogen which may lead to the discovery of potent antibiotic. The potency of the other extracts of *A. calamus* against bacterial strains was not good enough. This showed that the active components of *A. calamus* are more soluble in n-hexane. The presence of saponins in *A. calamus*, shown by its dichloromethane and ethanolic extract is the cause of hyperlipdemia in rats, while diarrhea does not occur with the large dose of the extract with ethanol. Alcoholic extract is responsible for anti-inflammatory effect, while n-hexane for spasmolytic effect of the plant. The presence of flavonoids in dichloromethane extract is responsible for showing antibacterial activity, particularly, against *S. aureus*. Ethyl acetate extract showed 14 mm zone of inhibition against *S. epidermidis*, whose phytochemical screening showed the presence of alkaloids, tannins and terpenoids. This showed that out of these three constituents, one, two or all are responsible for its

Table 1. Secondary metabolites detected in different extracts of *Acorus calamus*.

Chemical component	n-Hexane	DCM	Ethyl acetate	Ethanol
Alkaloids	+	++	++	+++
Tannins	-	-	++	++
Anthraquinones	-	-	-	-
Glycosides	-	-	-	++
Reducing sugar	-	-	-	++
Saponins	-	+	-	+++
Flavonoids	-	+	-	+
Phlobatanins	-	-	-	-
Terpenoids	++	+++	++	++

+++ = High concentration; ++ = Moderate concentration; + = Low concentration, - = Absent

Table 2. Secondary metabolites detected in different extracts of *Ferula asafetida*.

Chemical component	n-Hexane	DCM	Ethyl acetate	Ethanol
Alkaloids	++	++	+++	+++
Tannins	-	-	-	+
Anthraquinones	-	-	-	-
Glycosides	-	-	-	++
Reducing sugar	-	-	-	+
Saponins	-	++	-	+++
Flavonoids	-	-	-	++
Phlobatanins	-	-	-	-
Terpenoids	++	+++	++	++

+++ = High concentration; ++ = Moderate concentration; + = Low concentration, - = absent

Table 3. Antibacterial activity of *A. calamus* and *F. asafetida*.

Extraction solvent	Inhibition zone (mm)*					Inhibition zone (mm)*				
	<i>A. calamus</i>	E.C	S.E	S.A	B.S	<i>F. asafetida</i>	E.C	S.E	S.A	B.S
Hexane	Extract	0	0	12	10	Extract	12	16	0	12
Dichloromethane	Extract	14	12	0	15	Extract	12	0	8	10
Ethyl acetate	Extract	10	0	12	12	Extract	0	14	10	12
Ethanol	Extract	12	12	0	10	Extract	0	10	0	0
Streptomycin	1 mg/ml	24	36	34	30	1 mg/ml	24	36	34	30

E.C.: *Escherichia coli*; S.E.: *Staphylococcus epidermidis*; S.A.: *Staphylococcus aureus*; B.S.: *Bacillus subtilis*.

antibacterial activity.

Conclusion

The antimicrobial effects of *A. calamus* and *F. asafetida* extracts against the studied bacteria suggest that, different parts of *A. calamus* and *F. asafetida* possess

remarkable therapeutic action that can support the traditional usage of this plant in the treatment of bacterial diseases such as gastrointestinal infection, diarrhea, respiratory and skin diseases. These antimicrobial activities are likely due to the presence of secondary metabolites like tannins, flavonoids, alkaloids, saponins, terpenes and glycosides in *A. calamus* and *F. asafetida*. The high potency of *A. calamus* and *F. asafetida* against

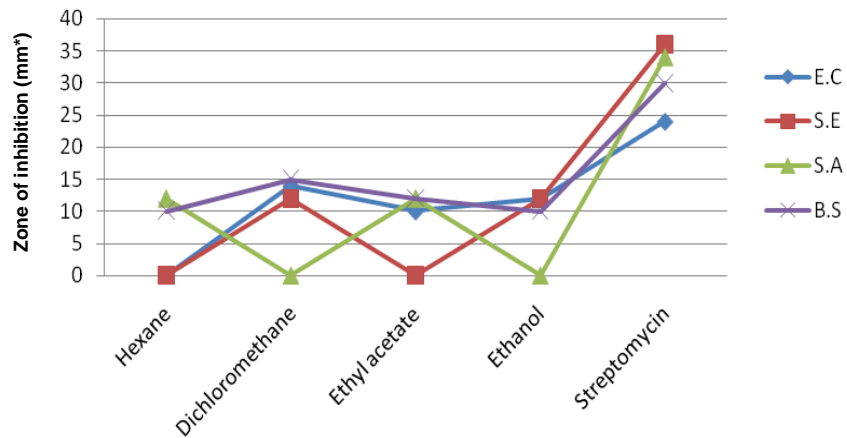


Figure 1. Antibacterial activities of *Acorus calamus* in different solvent.

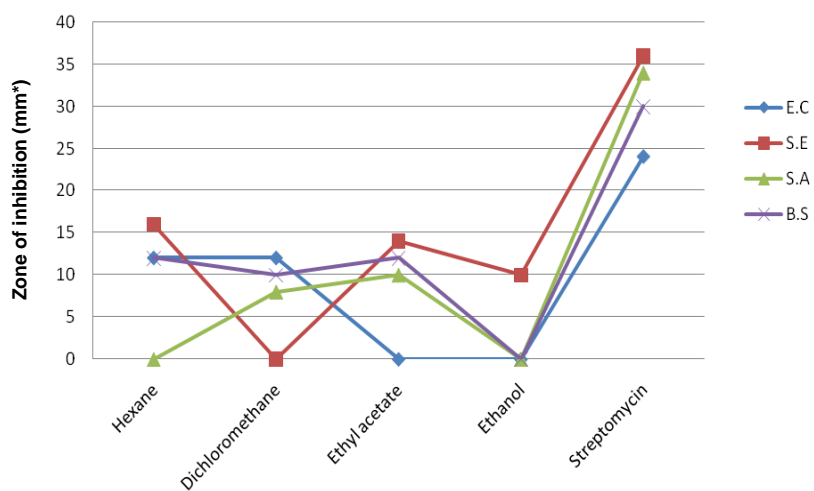


Figure 2. Antibacterial activities of *Ferula assafoetida* in different solvents.

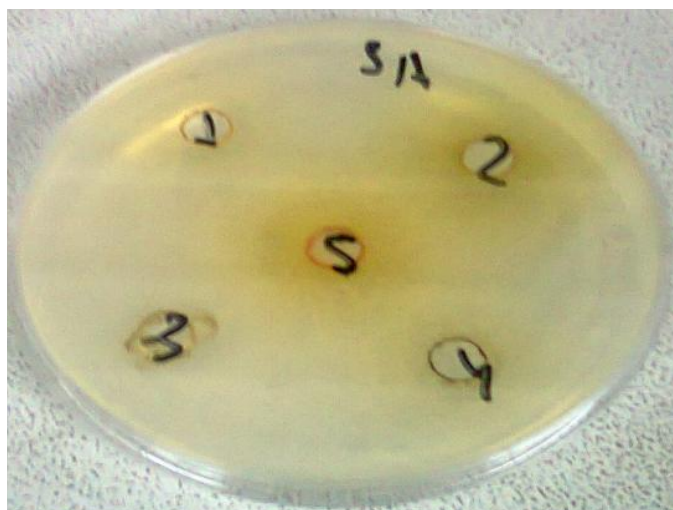


Figure 3. Cup-plate diffusion method for ethanolic extracts of the fruits and seeds against *Staphylococcus aureus*.

these microbes could provide an example of prospecting for new compounds.

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