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Antibacterial Activity of Ferula Asafoetida against Wound Pathogens and Its Applications in Wound Healing with the Extracts-Coated Guaze Dressing Material

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ABSTRACT
Infectious diseases can be caused by several different classes of pathogenic organisms .These are viruses, bacteria, protozoa, fungi. Almost all of these organisms are microscopic in size and are often referred to as microbes or microorganisms. Although microbes can be agents of infection, most microbes do not cause disease in humans. In this case the study helps to Extract the Ferula asafoetida in different organic solvents and check for its antimicrobial activity against the isolated organisms. The wound infection organisms from the infected pus samples were collected and thus It includes both bacterial and fungal culture strains which then streaked directly into the nutrient agar plates, then kept for incubation at 37ºC. Whereas, the morphological studies of isolated bacterial colonies were examined by gram'sreactiontodifferentiatebacteriaGrampositiveornegativeonthebasisof standard staining protocol. Motility testwas detected using hanging drop technique to determine the bacteria's motility. In this Biochemical test where examined for the identification of bacterial species based on the difference in biochemical activities of different bacteria. The characterization of the isolated bacteria was done by observing the colony morphology in the nutrient agar plates and in the respective selective mediums.Fungal isolates from wound infection (pus)samples were isolated and observed by LPCB staining test. The present study mainly analyses both the antibacterial and antifungal activity of Ferula asafoetida crude extracts in different inorganic solvents against the wound infection causing organisms. The different solvents extracts of Ferula asafoetida shows a better antibacterial activity. As it shows much antibacterial activity than the antibacterial action of pure inorganic solvents, it can be used as a source of new antibiotic compounds. When the crude extracts was coated on the gauze material again it shows antibacterial activity (AATCC Test) and the bacterial inhibition proves that the functionalized gauze fabrics prepared in the experiment has good antibacterial activity against the wound pathogens; it also can be used as a good source of wound healing substance. The crude extracts shows better antibacterial activity because of the dissolved bioactive compounds like secondary metabolites in it, which is present at the time of extraction.
Keywords:*Antibacterial, Asafoetida, Wound healing, crude extracts*

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INTRODUTION

Infectious diseases can be caused by several different classes of pathogenic organisms (commonly called germs). These are viruses, bacteria, protozoa, fungi. Almost all of these organisms are microscopic in size and are often referred to as microbes or microorganisms. Pathogens are usually distinct from the normal flora. They have developed highly specialized mechanisms for crossing cellular and biochemical barriers and for eliciting specific responses from the host organism that contribute to the survival and multiplication of the pathogen [1]. Only a minority of bacterial species have developed the ability to cause disease in humans. Some of those that do cause disease can only replicate inside the cells of the human body and are called obligate pathogens. Genes that contribute to the ability of an organism to cause disease are called virulence genes. The proteins they encode are called virulence factors. Virulence genes are frequently clustered together, either in groups on the bacterial chromosome called pathogenicity islands or on extrachromosomal virulence plasmids, these genes may also be carried on mobile bacteriophages. Some pathogenic bacteria use several independent mechanisms to cause toxicity to the

cells of their host [2]. Fungal infections are broadly divided as environmentally or endogenously acquired. For example, *Aspergillus fumigatus* grows as a saprophyte in environmental niches such as compost and soil and patients become infected via inhalation of spores. In contrast, *Candida albicans* is a member of the human microbiota and invasive infections typically originate from colonizing cells of the patient's own gastro intestinal tract. Therefore, these different human fungal pathogens have evolved pathogenic potential independently, either in environmental niches or as colonizers of mucosal surfaces pathogenic fungi are responsible for many diseases both in plants and animals and the identification of genes that are critical for the growth or those that may be targeted by anti- fungal drugs is of extreme social and economical importance [3]. Wound contaminants are likely to originate from three main sources, the environment, the surrounding skin, endogenous sources involving mucous membranes. However, to date widespread opinion among wound care practitioners such as *pseudomonas aeroginosa, staphylococcus aureus,* and beta haemolytic *streptococci* are the primary causes of healing and infection in both acute and chronic wounds[4]. The failure to recognize the prevalence of anaerobic bacteria in wounds may be due to several reasons are anaerobes are not regarded as being detrimental to normal wound healingand compared with aerobic and facultative microorganisms, the culture, isolation, and identification of anaerobic bacteria is more time-consuming, labor- intensive, and expensive and is often deemed to be too demanding for many diagnostic microbiology laboratories however, many of the frequent wound colonizers, including *bacteroides, prevotella, porphyromonas*, and *peptostreptococcus* spp, will survive for several days in the presence of air [5]. Characteristic local responses are a purulent discharge or painful spreading erythema indicative of cellulitis around a wound .

Ferula assafoetida is the plant utilized for manufacture of dried latex (gum oleoresin) which is exuded from the rhizome and stems of this plant belonging to the family Umbelliferae. A milky secretion exudes from the cut surface of rhizome, stems and the dried exudates are scraped off. The plant grows 1-1.5 m tall and possesses extremely dissected leaves the inconspicuous yellow flowers have been kept in compound umbels. The bark is black and wrinkled which contains great amounts of gelatinous alliaceous juice [6]. It is used in the treatment of various diseases such as intestinal parasites, flatulence, influenza, epilepsy, stomachache, asthma, and weak digestion. The pharmacological studies have demonstrated its antioxidant, antifungal and antimicrobial properties .

MATERIAL AND METHODS

Sample collection and culture maintenance

The wound infection organisms from the infected pus samples were collected from the multispecialty hospital at Coimbatore, during the first week of January 2020 and transported directly to the microbiology laboratory. It includes both bacterial and fungal culture strains which then streaked directly into the nutrient agar plates, then kept for incubation at 37ºC and the fungal strain were subcultured in the SDA agar plates kept in incubation for 2-5 days. The colonies from the plates were inoculated in freshly prepared nutrient agar broth and incubated at 37ºC for 24 hours in order to get the pure culture, this nutrient broth was used for the further study. Isolates were identified by based on Colony Morphology[7] on different culture media such as the nutrient agar medium, blood agar, MSA (Mannitol Salt Agar) and Cetrimide agar,gram staining technique, hanging drop technique to determine whether the bacteria are motile or non-motile and various biochemical tests were also performed.

Fungal isolates from wound infection (pus) samples from hospital were subcultured into the SDA plates by stabbing the culture at its center. And kept at room temperature for 2-5 day.The lactophenol cotton blue wet mount with gently teased the specimens were observed for the morphological identification of fungi.

For the extraction of *ferula asafoetida* powder in different solvents like petroleum ether, chloroform, methanol by using soxhlet apparatus [8] The commercially available powder of *Ferula asafoetida* (TT company) were taken into 50 gms to make the bag, which is made up of whatman No:1 filter paper (timple). The timple were placed in the extraction of the apparatus. The petroleum ether solvent were added in the round bottom flask of the apparatus. The in and out flow of water is maintained properly. The condenser of the apparatus always regulates the temperature. The apparatus runs for 3-4 hours continuously for gaining the organic solvent extract of the compound. Later the extraction is collected and kept for evaporation of the compounds. The process continues for the other two solvents such as chloroform and methanol and the extracts are collected and kept for evaporation.

The antibacterial activity of the extracts of *Ferula asafoetida* [9]was evaluated by agar well diffusion method. The sterile nutrient agar plates were prepared. The respective culture of *Staphylococcus sp., Pseudomonas sp., klebsiella sp.,* and a fungal culture of *Candida sp.,* were swabbed over the surface of the agar plates. Then 5 wells were made using a well cutter in a plate among the 5 one of the well was kept as control (The control used here is the solvents alone (without the compound) which is used for the

extraction). The crude extracts of *Ferula asafoetida* in petroleum ether was added in the concentration rangingfrom 20µl to 100µl. The concentration of the control is corresponding to one of the concentrations in other wells with the crude extracts. The process continued for the other two solvents of extracts such as chloroform and methanol. Then all the plates were kept for incubation at 37ºc for 24 hours. The zone diameters of each plates is observed and measured for the comparison of the Antimicrobial activity of the solvents with crude extracts of the compound with the antimicrobial activity of the solvents alone [10]. The Gauze wound dressing material was sterilized and it have been cut into small pieces. It was coated with the extracts of different solvents.Then it was kept for drying

The sterile nutrient agar plates, the test organisms (cultures of *Staphylococcus sp., Pseudomonas sp., Klebsiella sp.,)* in a liquid culture medium were prepared for the antibacterial activity of the coated fabrics against the wound pathogens (AATCC 147 Test Method). Using a sterile inoculating loop, one loopfull of the inoculums suspension is used to streak 5 consecutive steaks, spaced evenly apart, without refilling the loop, onto the solidified nutrient agar. This allows for 5 parallel streaks varying in concentration. The Gauze material have been cut to be rectangular in shape and measuring 25*50 mm, as recommended by the method, are evenly placed across the five parallel streaks.The furnishing of the material is done by coating with the crude extracts.The uncoated test sample is placed over the nutrient agar plate as control.Gentle pressure is placed on the coated material onto the agar in order to ensure contact with the test organism. All the plates is then kept for incubation at 37ºc for 24 hours.

Antifungal activity of the identified organism against the solvent extracts of *Ferula asafoetida*was checked on SDA plates by swabbing the plates with the fungal suspension using sterile swabs.After that the well wee punctured in the SDA plates and filled with 100µl of the previously prepared extract samples.The plates were kept for incubation at 28±ºC.The zone of inhibition is examined by measuring the diameter of the inhibition of the fungal mycelia growth.

RESULTS AND DISCUSSION

After isolation of the sample culture was subcultured into Nutrient agar plates and nutrient broth, each of isolated strain shows different colony morphology in the NA plates and in nutrient broth it shows the turbidity which indicates the growth of the organism , this nutrient broth was used for the further study. The morphological characteristics shown in the NA plates were observed and identified

Fig 3.1 Growth of *Klebsiella sp., Staphyloccus aureus*& *pseudomonas sp.,* in nutrient agar

The gram's staining showed that among the three types of colony morphology, two of them were observed as Gram negative rods and one was observed as Gram positive cocci. The Gram positive organism appear as purple color cocci and which is found to be as *Staphylococcus sp,* and the Gram negative organism appear to be as pink color rods and it is found to be as *Klebsiella sp* and *Pseudomonas sp.*

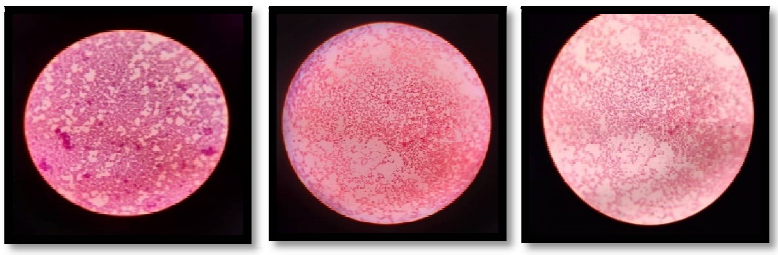

Fig 3.2. Gram staining result of three different isolates

Fig 3.3. Biochemical characteristics of *pseudomonas sp. & Klebsiella sp.*

Both the Gram negative rods showed the absence of cherry red ring indicated the negative result for indole production test, they also showed negative results for methyl red test which indicates absence of acidic end product, one among the Gram negative rods showed positive coloration for voges proskauer test (pink coloration) indicates the presence of alkaline end product, both of them shows positive result for citrate utilization test (Deep Prussian blue color)indicates that it can use citrate as sole source of carbon. Each of the organisms showed positive result for the catalase enzyme. One of the organisms showed positive result for the oxidase disc test (presence of the purple color) indicates that it is able to produce cytochrome oxidase enzyme. One of the isolated organisms shows coagulase positive indicates the presence of coagulase enzyme –like protein and causes plasma to clot by converting fibrinogen to fibrin.

In the lactophenol staining , the yeast like cells appears to be white opaque and oval in shape. The primary dictation shows the organism can be of *Candida sp.* The Lactophenol cotton blue stain shows the deep blue, round and unilateral budding yeast cells.

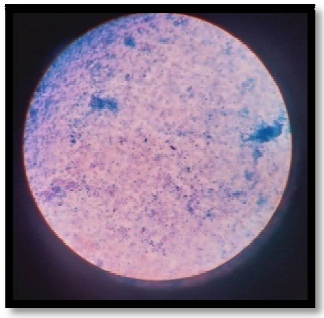

Fig 3.4. Lactophenol cotton blue staining of *Candida sp*

Extraction of the compounds of *Ferula asafoetida* using three different solvents. The extracts were kept for evaporation, after evaporation the amount of the final extract has been weighed and makeup into 25 ml each with respective solvents. The antimicrobial activity of the extracts of *Ferula asafoetida*, the organisms like Staphylococcus *sp.*, Klebsiella *sp.,* Pseudomonas sp., shows more resistance against the methanolic extracts of *Ferula asafoetida* than the extracts of the compound in the petroleum ether and chloroform. The organisms against the petroleum ether extracts also shows zone of inhibition very least but the organism against the chloroform extracts doesn't shown any zone of incubation.

Fig 3.5 The Antibacterial activity of *Ferula asafoetida* petroleum ether extract against wound pathogens

Table 3.6.1 Antibacterial activity observed by using petroleum ether extracts of *Ferula asafoetida.*

Sl No	Organism	Zone of inhibition (mm)				
		20µl	**40µl**	**80µl**	**100 µl**	**Concentration of pure solvent (C)20 µl**
1.	*Staphylococcus aureus*	29mm	31mm	34mm	38mm	25mm
2.	*Klebsiella sp*	23mm	25mm	30mm	33mm	21mm
3.	*Pseudomonas sp*	25mm	28mm	31mm	34mm	21mm

Fig 3.6 The Antibacterial activity of *Ferula asafoetida* Methanol extract against wound pathogens.

Table 3.6.2 Antibacterial activity observed by using methanolic extracts of *Ferula asafoetida.*

Sl No	Organism	Zone of inhibition(nm)				
		20µl	40µl	80µl	100µl	Concentration of pure solvent (C) 20µl
1.	*Staphylococcus aureus*	28mm	30mm	35mm	41mm	21mm
2.	*Klebsiella sp*	15mm	18mm	30mm	34mm	13mm
3.	*Pseudomonas sp*	20mm	24mm	38mm	31mm	20mm

The methanolic extract of the Ferula asafoetida compound shows higher activity against the wound organisms. The control was used as the same solvents, the crude extracts shows more zone diameter than compared to the action of the solvent alone. The petroleum ether extract and the chloroform extract shows least zone of inhibition may be because of the lack of diffusion of the extracts.

Comparison of the antimicrobial action of the crude extracts of *Ferula asafoetida* with the antimicrobial action of the pure organic solvents like Petroleum ether, Chloroform and the methanol; The organisms shows more resistance against the crude extracts of *Ferula asafoetida* in the agar well diffusion method than the resistance shown by the organisms in the pure solvents itself. The organisms shows more zone of inhibition in the methanolic extract of *Ferula asafoetida* than the chloroform and petroleum ether extracts .The zone diameter shown by the organisms against the control solvents is comparatively less than the action of the crude extracts.

The crude extracts of the herbal products shows more activity against the wound organisms it may due to the bioactive compounds present in it. The organisms shows more resistance by the action of methanolic extract of the asafoetida compound. The methanolic extract has significant antimicrobial activity due to the occurrence of a mixture of phytoconstituents and it could be a source of new antibiotic compounds.

For the Furnishing of gauze material with the respective extracts of *Ferula asafoetida,* the gauze wound dressing material was coated with the extracts of asafoetida and kept for drying. It was then used for testing the antibacterial activity of gauze material coated with the extracts against the wound organisms. The materials allows the absorbance of the extract easier the material allows egress of fluid and bacteria through the mesh. As the dressing dries, fibrin from the wound bed causes temporary bonding of the dressing to the wound. It has the debridement action and it provides the bacterial protection more.

The organisms showed more zone of inhibition in the petroleum ether extracts, around the coated material in the agar plate. The other two solvent extracts does not showed an effective result against the bacteria in the antibacterial activity of the coated gauze material against the wound pathogens.
This test tests the ability of the treated textile to inhibit the growth of the microorganisms, to be bacteriostatic. It estimates the activity and efficacy against different concentration of the microorganisms. The petroleum ether extracts showed an effective result against the activity of the organisms in different concentrations. The other two solvents does not showed an effective result may be because of the lack of diffusion of the extract.

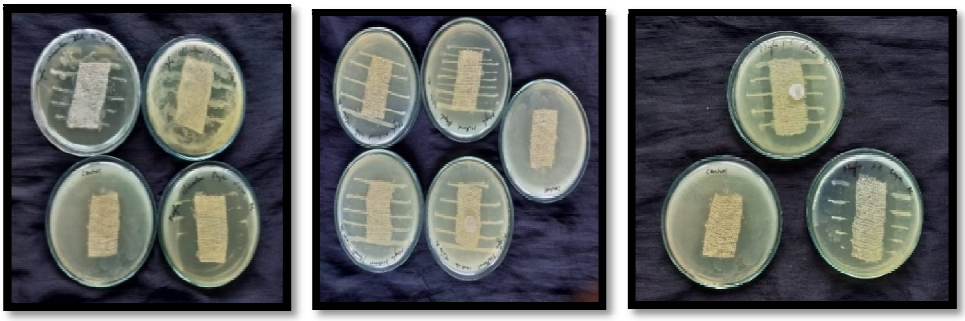

Fig 3.7. The Antibacterial activity of the gauze material coated with Petroleum ether, chloroform and Methanol against the wound pathogens.

Antifungal activity of *Ferula asafoetida* Extracts; The Identified fungal sp of *Candida* does not show any antifungal activity in three of the solvent extracts of *Ferula asafoetida.* It is may be because of lack of diffusion of the extracts.

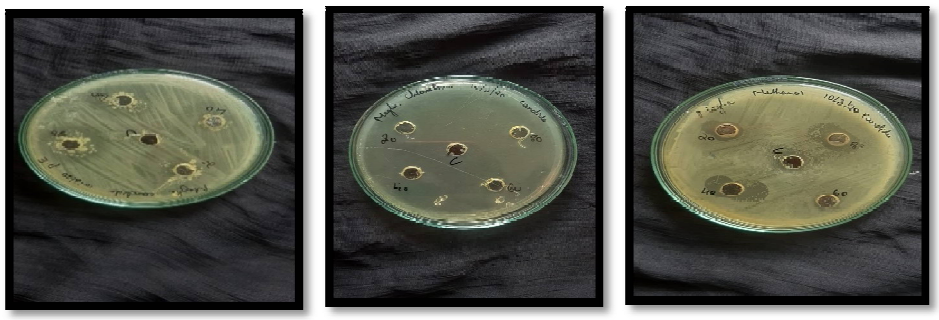

Fig 3.8. The Antifungal activity of the gauze material coated with Petroleum ether, chloroform and Methanol against the wound pathogens.

CONCLUSTION

In the present study, use of various herbs and traditional medicine is safe as well as economical in the present scenario of escalating health care cost. Herbal therapy acts thus as a much cheaper, it may suffer and most widely accepted concept of humanity throughout globe, possessing multi-dimensional health benefits. However, the traditional medicinal plants require exhaustive scientific validation as well as standardization and safety evaluation before they can be accepted for commercialization.
In the study, the wound pathogens like *Staphylococcus sp, klebsiella sp, pseudomonas sp* showed resistance against the extracts of *Ferula asafoetida* in methanol more than that of other two extracts in chloroform and petroleum ether. The extract with the petroleum ether shows less resistance against the pathogens and chloroform doesn't shown a significant resistance against the pathogens. These may be due to the lack of diffusion of the extract. The antimicrobial activity of the crude extracts of *Ferula asafoetida*in solvents were compared with that of the action of the same pure solvents alone. The action of the crude extract with the compound seemed to show more zone of inhibition than that of the zone of inhibition of the solvent alone. The antibacterial activity of the coated wound gauze material by AATCC 147 test method, the organisms showed less resistance against the coated fabrics in methanol and chloroform. The antibacterial activity of the petroleum ether coated fabrics showed significant resistance against the wound organisms. By this present study we can conclude that the methanolic extract has significant antimicrobial activity due to the occurrence of a mixture of phytoconstituents and it could be a source of

new antibiotic compounds. The herbal traditional medicine will always aids for a good health for the individuals without any side effects because of the presence of the bioactive compounds in it.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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