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Antibacterial activity of *Ferula asafoetida:* a comparison of red and white type

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ABSTRACT

Ferula asafoetida, a native species of Iran and Pakistan has a broad spectrum antimicrobial property in addition to its household use. It has been used as an analgesic agent from the time of Charak Samhita. In the present study we used two kind of *F. assafoetida* (red and white) gum to screen their antimicrobial activity against five different bacterial strains. Extracts of both types of assafoetida were prepared in organic and inorganic solvents. Polar extracts were found to have significantly higher antimicrobial activity than its nonpolar counterparts. Highest antibacterial activity was shown by hexane extract against *Shigella flexineri* and *S. aureus* was found to be least affected by these extracts. However, extracts of both red and white forms showed comparable antibacterial activities, so it may have same chemical composition. The difference in antibacterial spectra of different solvent extracts observed might be due to some other secondary metabolites that are soluble in organic solvent contributing varying antimicrobial property.

1. INTRODUCTION

The use of medicinal plants as a source of alleviation from diseases can be trailed back in the written documents of the early civilization in China, India and near east, but it is doubtless an art as old as mankind. Medicinal plants are used to cure the disease since ancient time. The use of medicinal plants in the world especially in developing country like India, contributes significantly to primary health care. It is well known that herbal medicine has a very large potential, which is insufficiently explored. Knowledge of medicinal plants is also sometimes thought to be the only therapeutic resource of some communities and ethnic groups.

The ability of plants to cure or prevent diseases is because of different phytochemicals present in them. These bioactive compounds of plants include alkaloids, flavonoids, tannins and phenolic compounds. Phytochemicals isolated from the medicinal plants show different antimicrobial activities. Therefore, these phytochemical have the capacity of filling the requirement of antimicrobial drugs. Phytochemicals differs from antibiotics in structure, so having different mode of action [1]. A number of studies have been done to assess antimicrobial activities of different plants from time to time [2-5]. Antibacterial drugs which are obtained from microbial source soon become ineffective due to development of resistance among bacteria. Antibiotics are sometimes also associated with adverse effects [6]. Development of drugs from plant origin and correlation of secondary metabolites with its pharmacological activity is a growing area of interest now a days [7]. Bacteria cannot develop resistance to these drugs easily. Screening of active compounds isolated from plants has lead to the discovery of new drugs which can be used more efficiently in protection and treatment of various diseases, including cancer also [8]. Natural products have proved to be an important source of new drugs. Screening of natural plant products provides the chance to find out the new molecule of unique structure with selectivity which can be used as antimicrobial drugs. Spices used our day to day cooking are not the exception. Spices are not important only in flavour adding but they also have medicinal properties [9-11]. There are number of reports available in literature describing the sensitivity of various bacteria towards spices used in food preparations [12]. Spices like turmeric and cloves are well known for their anti septic and wound healing properties, In addition, turmeric prevents Alzheimer's disease and cloves are commonly used as carminative agents [13]. Use of Assafoetida in counteracting intestinal flatulence is well supported in Indian cooking and treating colic pains in infants. Assafoetida (*Ferula asafoetida*) is a species of *Ferula* which grows mainly in Iran. It is an herbaceous plant with height of 2 ml, having short, hollow, succulent stems 5-8 cm diameter at the base of the plant. It is commonly known as Hing or Devils dung.

In pure form it has very pungent smell. In market it generally available in mixture with rice or wheat flour which is used in kitchen. Hing is extracted as the resin from roots of its plant. It also reduces flatulence. The species are distributed from the Mediterranean region to Central Asia. In India it is grown in Kashmir and in some parts of Punjab.

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It is a native of Afghanistan and Iran. Its taste is bitter and has a strong pungent smell. Asafoetida contains about resin (40-64%), endogenous gum (25%) , volatile oil (10-17%) , and ash (1.5-10%). There are two main varieties of assafoetida are based on colour i.e. Hinge Kabuli Sufaid (Milky white assafoetida) is water soluble and Hinge Lal (Red assafoetida) which is oil soluble. In the present study we checked and compared both the varieties of hinge for their antibacterial activity. The antibacterial activity of *F. assafoetida* extracts were evaluated against five different bacterial strains (Table-1). All the bacterial strains studied were aerobic except one i.e. *Enterococcus faecalis* which exhibit facultative anaerobic respiration. Among the bacteria studied *E. coli* and *K. pneumonia*, *Sh. flexneri* are Gram negative whereas *S. aureus* and *Enterococcus faecalis* are Gram positive.

Table 1: Represent the diameter of zone of inhibition of different extracts against the tested bacterial strains.

Name of extract		Zone of inhibition in centimeter				
		E.coli	***S.aureus***	***K. pneu***	***S. flex***	***E. faecaelis***
Petroleum ether	Red	1.0	0.9	1.0	1.3	0.8
	White	0.8	0.7	0.7	1.3	0.8
Hexane	R	0.9	0.8	1.2	1.7	0.7
	W	0.8	1.1	0.9	1.5	0.7
Hot water	R	1.1	-	0.8	0.7	0.7
	W	1.2	_	0.8	0.7	0.9
Cold water	R	0.9	0.7	0.8	0.8	1.2
	W	1.0	_	0.9	0.8	0.9
Ethanol	R	1.2	0.8	0.9	1.2	0.9
	W	0.7	0.7	0.8	1.1	0.7
DMSO	R	—	—	—	–	—
	W	—	—	–	–	—
Amoxicillin	R	2.9	2.9	1.3	2.7	1.7

2. MATERIALS AND METHODS

2.1 Collection of plant material and Extract preparation

Red and white forms of *F. assafoetida* were purchased from the local market. Both the types were firstly dried and then grinded to powder. 10g of both powdered forms was suspended in 100ml autoclaved distilled water. Flasks were kept on shaker for 48h. Solution was filtered and filtrate was dissolved in DMSO. Similarly the extracts in hot water, hexane, ethanol and petroleum ether were prepared

2.2 Revival of strains

Strains were purchased from IMTECH, Chandigarh. Lyophilised form of strains were added in to the small amount of LB broth. Broth was then incubated at 37^0 C for 48 h. Streaking was done on slant by taking a loop full of bacterial culture and allowed to incubate at 37^oC for 48h, and then slants were stored at -4^oC for future use.

2.3 Determination of antibacterial activity

Antibacterial activitiy of the different extracts of *F. assafoetida* was determined by disc diffusion method [14]. Nutrient agar was used as antibacterial susceptibility test medium. Filter paper discs of 6mm size were soaked in different extracts and allowed to dry. Bacterial strain was subjected overnight incubation in peptone water for inoculum preparation. The turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 CFU/ml for bacteria).

Then bacterial suspension was spread on the entire surface of agar plate and dried discs were placed on it. Disc of DMSO was used as the negative control and disc of antibiotic Amoxicillin was used as positive control. Plates were placed for 1-2 h at 4 ^oC for diffusion of extracts properly to form a concentration gradient of extract uniformly around the disc on agar plates before the growth of bacteria and then the plates were incubated at 37 ^oC for 24h. Zone of inhibition was measured with help of transparent scale.

3. RESULTS AND DISCUSSION

Microbes are the main agent causing human health problems. Chemical based antimicrobial therapy definitely kills the microbes but also affects the host health. Medicinal plants are the valuable commodity of commerce and herbal medicines, gaining momentum for a wide variety of human ailments due to the high cost of treatments by allopathic drugs, their side effects and the development of resistance against antibiotics. People who were away from the conventional systems of medicine are alluring towards green pharmaceuticals as the period of synthetic drugs has almost dwindled, and drugs derived from plants have integrated in formal health care systems.

The plant, *F. assafoetida* is well known for its use in not only ayurvedic & traditional system of medicine but also as a day to day used household spice that is known for its digestive properties.

Various extracts of this plant were prepared and screened for their antimicrobial activity. Five different bacterial strains were selected for the study comprising both Gram positive as well as Gram Negative bacteria (*Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Shigella flexneri, and Klebsiella pneumonia)*. Except *Staphylococcus aureus*, all other bacteria are related with gastrointestinal infections. Amoxicillin, which was used as positive control showed considerable activity against all bacteria. DMSO was used as negative control. All the extract of the *F. assafoetida* (both red and white) showed strong antibacterial activity against all the bacterial strains. Antibacterial activity of the extracts was assessed by disc diffusion method.

The various extract of the medicinal plant *F. assafoetida* showed strong antibacterial activity against the tested bacteria, their zone of inhibition is shown in Table-1. All organic extracts showed greater zone of inhibition in comparison to the aqueous extracts (fig 1). Highest zone of inhibition was observed in case of Hexane extracts. Aqueous extracts showed their maximum activity against the *E. coli* and it was found that hot water extract was more active. Antibacterial activity against *S. aureus* was minimum in case of all the extracts prepared and maximum against *Sh. flexneri.* DMSO exhibited very less or no activity against bacteria.

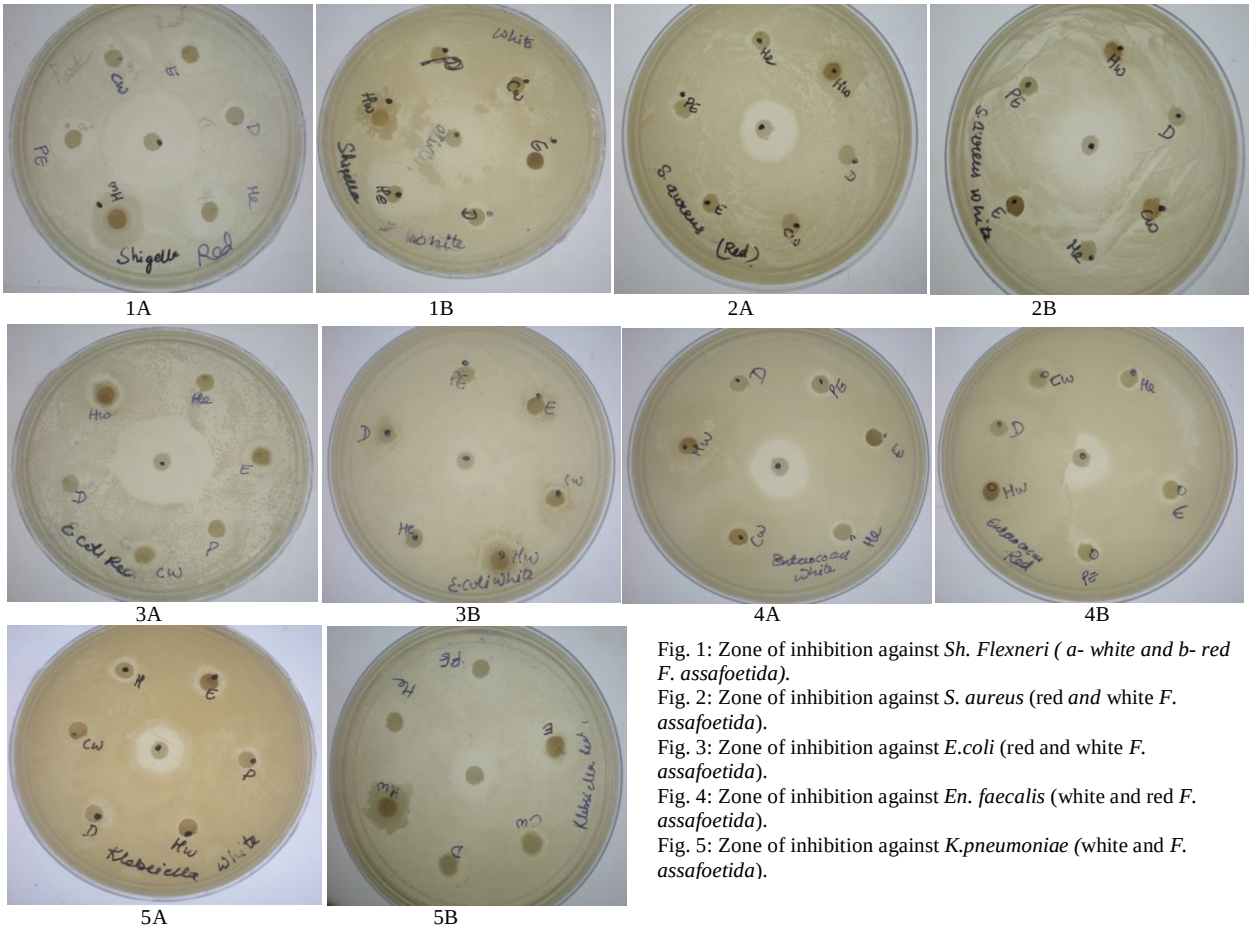

Fig. 1: Zone of inhibition against *Sh. Flexneri (a- white and b- red F. assafoetida).*
Fig. 2: Zone of inhibition against *S. aureus* (red *and* white *F. assafoetida*).
Fig. 3: Zone of inhibition against *E.coli* (red and white *F. assafoetida*).
Fig. 4: Zone of inhibition against *En. faecalis* (white and red *F. assafoetida*).
Fig. 5: Zone of inhibition against *K.pneumoniae (*white and *F. assafoetida*).

In present investigations no difference in the antibacterial activity of red and white types of assafoteida was observed however, more activity of their organic extracts gives a clear indication that further refinement may be more beneficial in organic solvents and the effects may be more pronounced against enterobacters.

4. CONCLUSION

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of diseases caused by microbes. From our studies, we conclude that extracts of *F. assafoetida* demonstrated activity against both gram-positive and gram-negative bacteria. However, enterobacters were found to be more susceptible. Organic extracts has shown stronger activity in comparison to aqueous extracts. *F. assafoetida* has broad-spectrum antibacterial activity because it showed the good inhibitory potential against the tested strains of bacteria. This confirms its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial infection mainly related to the digestive system. Isolation, identification and purification of theses phyto-constituents from more pure components of *F. assafoetida* and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents can be the future direction for investigation.

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