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Antimicrobial activities of *Asafoetida* resin extracts (A Potential Indian Spice)

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ABSTRACT

Indian herbs and spices are known for their preservative, flavouring and medicinal values. The main aim of this study is to strengthen the multiple potential values of *Asafoetida*. In this study, Antimicrobial activities of crude extracts of *Asafoetida* were evaluated against different bacterial and fungal strains. It was observed that alcoholic and aqueous extracts of *Asafoetida* showed significant effect against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* and *Aspergillus niger* by agar disc diffusion method. The crude extract showed a broad spectrum of antimicrobial activities by inhibiting the respective bacteria and fungi. Agar disc diffusion assay for antimicrobial activity yielded the inhibitory zone of 4 to 16 mm diameter for *Asafoetida* extracts. The present study supports the immense medicinal properties of *Asafoetida*.

Keywords: Anitimicrobial activity- Agar disc diffusion- inhibitory zone-Medicinal Potential

INTRODUCTION

Natural products and their derivatives have historically been valuable as a source of therapeutic agents. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and are used in the traditional systems of medicine (Iwu, 1993). Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources (Parekh and Chanda, 2007). It has been reported that between the years 1983 and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi resistant bacteria. According to recent studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine. Indian herbs and spices are recognized to prevent the microbial deterioration of food. Herbs and spices have been used for thousands of years to enhance the flavor, colour and aroma of food. In addition to boosting flavour, herbs and spices are also known for their preservative and medicinal values, which forms one of the oldest sciences (Nielsen and Rios,2000). A large number of plants are used to combat different diseases and possess antimicrobial activity (Kalia *et al.*, 1977; Rusia and Srivastava, 1988; Schneider and Kubelka, 1989; Ayoub, 1989; Gangrade *et al*., 1990; Mahajan *et al*., 1991; Arora and Ohlan, 1997; Arora and Bhardwaj, 1997 and Arora, 1998). *F. assafoetida L*. is a herbaceous wild plant, native to Iran. It is herbaceous and perennial and grows up to 2 m high. Houghton *et al*., (2006) reported antifungal activity of *Asafoetida* against *Microsporeum gypseum* and *Trichophyton interdigitale*. Thyagaraia & Hosono (1996) also studied the inhibition effect of *Asafoetida* on *Rhizopus sporus*, *Mucor dimorphosphorous*, *Penicillium commune* and *Fusarium solani*. Antimicrobial activity of spices depends on several facts i.e. kind of spice, composition and concentration of spice and its occurrence level, substrate composition and processing condition and storage. Several scientific reports describe the inhibitory effect of spices and herbs on a variety of micro-organisms, although considerable variation of resistance of different micro-organisms to different spices has been observed. Recent data suggests that 80 % drug molecules are natural products or natural compounds. Natural products have long been a thriving source for the discovery of new drug due to their chemical diversity and ability to act on various biological targets. The photochemical exploration of indigenous flora has contributed to some extent in this race for the discovery of new drugs. Thus this review has been prepared with the aim to focus on the role of Indian medicinal plants in the global drug discovery process mainly in the disease areas like cardiovascular, viral, metabolic, stomach, inflammatory, parasitic. The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules. If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell et al. 1992). Many new drugs emerged by this route, particularly those now being used to treat infections, infestations, cancers, ulcers, heart and blood pressure conditions. Many drugs were developed through random screening of thousands of chemicals synthesized as dye-stuffs and many others resulted from serendipity (happy chance) arising from sharp-eyed observations of physicians and scientists. Examples of such drugs include sulphonamides, isoniazid, anti-psychotics, anti-histamines and penicillin. These successes and many more like them resulted in reduced interest in natural products drug discovery and many major drug companies almost neglected such divisions. Work on developing new drugs for the treatment of the world's major diseases, malaria, Trypanosomiasis filariasis, Tuberculosis, Schistosomiasis, Leshmaniasis and Amoebiasis came almost to a standstill. In addition, although botanical medications continued to be produced in every country, the clinical efficacy of these was usually not evaluated and the composition of these complex mixtures was only crudely analysed. Thus, herbal medicines became the domain of 'old wives' tales' and quack medicine, exploitation of the sick, the desperate and the gullible. *Asafoetida* is a spice and herbal medicine used to treat nervousness, bronchitis and gas pain. There are number of reports available in literature stating the sensitivity of various bacteria and fungi towards the asafoetida leaves, stems and oil. Most of work done involved extraction of leaves, stems of *Asafoetida*. But no more work has been done on *Asafoetida* resins. Resin is that substance which is leaked out from the plant cracking (Fatehi, 2004).

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MATERIALS AND METHODS

Collection of sample

Resin or exudates of the *Asafoetida* plant was used as a sample for experi-

ment. For this experiment crude form of *Asafoetida* was used. All the chemicals, reagents and media used for investigation were of analytical grade.

Chemicals and solvents
Ethanol (Rankem, India), Acetone (Rankem, India), Petroleum ether (Qualigen, India), Carbon tetra chloride (Rankem, India) with methanol (Merck) and Distilled water were used for the extraction of *Asafoetida*.

Media used For the experiments mainly two media were used.
1. Nutrient agar media (NAM) (Himedia, India)
2. Potato dextrose agar media (PDA) (Himedia, India)

METHODOLOGY

Preparation of extracts:
The crude form of *Asafoetida* was crushed into fine powder with the help of pestle and mortar. Then 5 grams of the crude (resin) form of *Asafoetida* was weighed on weighing machine. It was dissolved in 100 ml of ethanol. Then it was left for 24 hours in the shaking incubator. Sample was then centrifuged at 5000 rpm for 10 minutes and the supernatant was used as an extract. Similarly, petroleum ether, acetone, carbon tetrachloride, methanol and aqueous extracts were prepared.

Micro-organisms used
Bacterial and fungal strains were procured from Microbial Test Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, Punjab. The accession no. of bacterial strains was *E.coli* MTCC-443, *Pseudomonas aeruginosa* MTCC-4673, *Staphylococcus aureus* MTCC-3160, *Bacillus subtilis* MTCC-441, *Aspergillus niger* MTCC-1344. These were maintained on nutrient agar slants. All the isolates were sub cultured regularly in nutrient broth and slants and fungus was maintained on Potato Dextrose Agar at 28 °C.

Screening for antimicrobial activities

Antibacterial activity
For the determination of antibacterial activity agar disc diffusion method was used. Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer (1966) and Ncube *et al.* (2008). Different extracts were dissolved in their respective solvents. Pure solvent were used as control in the study. The inoculums for each microorganism were prepared from broth cultures. A loop of culture from the nutrient agar slant stock was cultured in LB medium overnight and spreaded with a sterile swab into petri-plates. Sterile disc (6 mm dia, Himedia, Mumbai, India) impregnated with the plant extracts (1 mg/ml, and 5 mg/ml) were placed on the cultured plates and incubated for 24 h at 37°C. The solvent loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate. Tetracycline was used as positive control and solvents were used as negative control.

Antifungal activity:
The compound exhibited significant antifungal activity against pathogenic strains of *Aspergillus species* and *Candida albicans* in disc diffusion assay. The activity was determined after 72 hours incubation at 28°C. The diameter of the inhibition zones were measured in mm.

RESULTS
The petroleum ether extract of *Asafoetida* crude sample showed antibacterial activity against *Pseudomonas aeruginosa* with inhibition zones of 6.4 mm and 16 mm against *E.coli*.(Table 1, **Fig1**) whereas there was no antibacterial activity against *Bacillus subtilis* and *Aspergillus niger.* The ethanolic extract of *Asafoetida* crude sample showed activity against *S.aureus* with inhibition zones of 11.7 mm (Table 1). Whereas there was no antibacterial activity against *Bacillus subtilis* and *Aspergillus niger.* However, ethanolic extracts showed a highest inhibition (17.1 mm) (Table 1, **Fig 2**) against *E.coli.* The acetone extract of *Asafoetida* crude sample showed activity against *E.coli* (**Fig 3**) and *Pseudomonas aeruginosa* with inhibition zones of 14.6 mm and 13.9 mm respectively and no antifungal activity (Table 1). The carbon tetrachloride and methanol extract of *Asafoetida* crude sample showed activity against *S.aureus* and *E.coli* (**Fig 4**) with inhibition zones of 4.9 mm and 9.2 mm respectively (Table 1). The carbon tetrachloride and methanol extracts did not showed activity against *Bacillus subtilis* and *Pseudomonas aeruginosa* and no antifungal activity. The aqueous extracts did not showed antibacterial or antifungal activity (Table 1).

Table no. 1: Antimicrobial Activity of *Asafoetida* crude sample

Extract	*S.aureus*	*E.coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Aspergillus niger*
Petroleum ether extract	6.3mm	16 mm	6.4 mm	-	-
Ethanol extract	11.7 mm	17.1 mm	4 mm	-	-
Acetone exract	13 mm	14.6 mm	13.9 mm	-	-
Carbon tetra chloride and methanol extract	4.9 mm	9.2 mm	-	-	-
Distilled water extraction	-	-	-	-	-

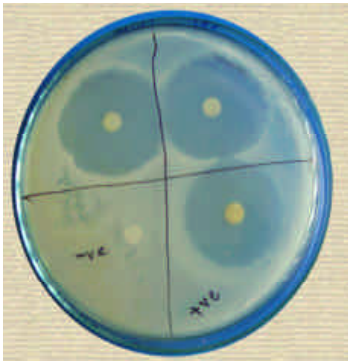

Fig. no. 1. Crude sample – Petroleum ether extract against *E.coli*

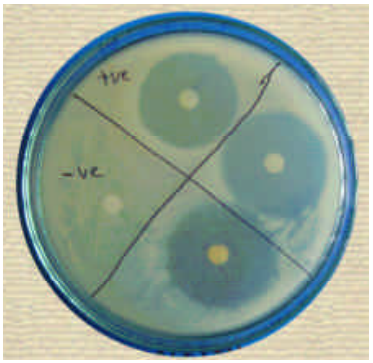

Fig. no. 2 Crude sample – ethanol extract against *E.coli*

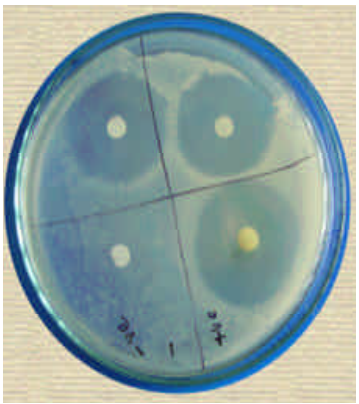
Fig. no. 3. Crude sample - Acetone extract against *E.coli*

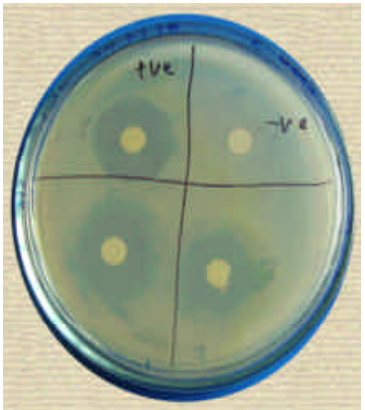

Fig. no. 4 Crude sample – CCl_4+ Methanol extract against *E.coli*

DISCUSSION
Natural products have always been a prolific source of new drugs and drug lead even from the vedic time. Even today, the world health organization estimates that up to 80% of people still relay mainly on traditional medi-

cines. The use of traditional medicine to treat infection has been in practice since the origin of mankind. Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The acetone, petroleum ether, ethanol, carbon tetrachloride with methanol of asafoetida crude sample were subjected to a preliminary screening for antimicrobial activity against *S.aureus* and *E.coli*. It was clear from the present results that asafoetida crude sample is less effective against *Pseudomonas aeruginosa* in comparison to the *S.aureus* and *E.coli*. *B.subtilis* and showed no inhibition zone against *A.niger*. So, *Asafoetida* crude sample is less effective against *B.subtilis* and *A.niger*. *Asafoetida* is commonly found in three forms, such as 'tears', 'mass' and 'paste'. The first being the tears which constitute the purest form of the resin and are rounded or flattened in shape, 5 to 30 mm in diameter and dull yellow or grayish in color. This spice is used as a digestive aid, in food as a condiment, in pickles, reduces the growth of indigenous microflora in the gut, reducing flatulence, efficient in preventing snake bites and repelling insects when mixed with garlic, relieve mucous and eases the respiratory system. India's ayurveda system recommends *Asafoetida* for the treatment of colic as it stimulates the mucus membrane in the body. It is also used in the treatment of high cholesterol and irritable bowel syndrome.

The present study has been undertaken to explore the antimicrobial properties of Asafoetida against various harmful human pathogenic bacteria. The study reveals that the spice is significantly active and can be proved worthwhile as a new drug discovery. Moreover the history reveals that Spices have been used earlier for their good pharmacological and physiological properties. Spices and Medicinal plants have always attracted great attention of researchers in India as well as worldwide for their beneficial health aspects. Thus by the present study *Asafoetida* can be recommended for therapeutic and medicinal purposes.

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