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EVALUATION OF ANTIMICROBIAL ACTIVITY OF ASAFOETIDA

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ABSTRACT: This study was carried out with the objective of the antibacterial and antifungal activity of chloroform, ethyl acetate, ethanol, methanol and aqueous extracts of Asafoetida. Antibacterial activity was carried out against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli* and the antifungal activity was evaluated against *Aspergillus niger*, *Candida albicans*. The testing was done by well diffusion method and evaluation was done by detecting zone of inhibition (in mm) and minimum inhibitory concentration (MIC). Studies were perfomed with two different concentrations of all extracts (2 mg/ml and 4 mg/ml). Zone of inhibition were compared with standards like Ciprofloxacin (0.1 mg/ml) and Fluconazole (0.1 mg/ml). The results showed that ethyl acetate, ethanol, and methanol extract has significant antimicrobial activity and highest activity was reported with methanolic extract. The minimum inhibitory concentration of methanolic extract, ethanolic extract and ethyl acetate extract against most of the test microorganisms were 1 mg/ml, 1 mg/ml, 2 mg/ml respectively.

INTRODUCTION: Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, Overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms [1]. The worldwide emergence of *Escherichia. coli*, *Klebsiella pneumoniae*, *Haemophilus* and many other ß-lactamase producers has become a major therapeutic problem.

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Multi-drug resistant strains of *E. coli* and *K. pneumoniae* are widely distributed in hospitals and are increasingly being isolated from community acquired infections [2, 3]. *Candida albicans*, also a nosocomial pathogen, has been reported to account for 50-70% cases of invasive candidiasis [4]. Alarmingly, the incidence of nosocomial candidemia has risen sharply in the last decade [5].

All this has resulted in severe consequences including increased cost of medicines and mortality of patients. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy [6]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains [7]. In all regions of the World, history shows that medicinal

plants have always held an important Place. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [8]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity [9]. There are several reports on the antimicrobial activity of different herbal extracts [10-12].

Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [13, 14]. According to the WHO, medicinal plants would be the best source for obtaining variety of drugs [15]. These evidences contribute to support and quantify the importance of screening natural products. The aim of the present study was to investigate the antibacterial and antifungal activity of different extracts of Asafoetida against different microbial strains.

Asafoetida: **Asafoetida** or **asafetida** is the dried latex (Oleogumresin) exuded from the rhizome or tap root of several species of *Ferula.*

- *Ferula assa-foetida*
- *Ferula caspica*
- *Ferula communis*
- *Ferula conocaula*
- *Ferula foetida*

Asafoetida

Phytochemistry

Asafoetida comprises of resin (40 to 65%), gum (20 to 25%) and volatile oil (4 to 20%).

The resin portion of asafoetida consist asaresinotannol as its key constituents in the free or combined form as esters of ferulic acid. Galbanic acid is also one of the widely present sesquiterpene in resin portion of the drug. One more key constituent of asafoetida is umbelliferone. The gum fraction consists mostly of glucose, galactose, 1-arabinose, rhamnose and glucuronic acid [16, 17]. The oil of asafoetida is usually obtained by steam distillation of oleo-gum resin. The oil comprises mostly of volatile polysulphides. Apart from the volatile polysulphides there are numerous terpenoidal compounds present additionally [18, 19].

Apart from the aforesaid sesquiterpenes and the volatile polysulphides the various other phytoconstituents are the diterpenes like the 7-oxocallitrisic acid, picealactone C, 15-hydroxy-6-en-dehydroabietic acid, various phenolic compounds like vanillin, 3,4-dimethoxycinnamyl-3-(3,4-diacetoxyphenyl) acrylate, acetylenes such as falccarinolone and various other miscellaneous compounds such as oleic acid and β-sitosterol [16, 18, 19].

Sesquiterpene coumarins such as foetidin, 4 methoxycoumarin, colladonin, asafoetidnol A, assafoetidnol B are the constituents present in the roots. Apart from the above mentioned sesquiterpene compounds the roots of *Ferula* species also consist of a volatile polysulphides derivatives, foetisulphide A and foetisulphideC [19, 20].

MATERIALS AND METHODS:

Plant Material

Asafoetida was collected from local market in Mumbai, Maharashtra. This powder was stored in an air tight container for successive extraction.

Preparation of Extracts

Chloroform, ethyl acetate, ethanol, methanol (95 %) and aqueous extracts of Asafoetida were prepared by maceration method. The powdered plant material (250 g) was repeatedly extracted in a 1000 ml conical flask with 500ml solvents of increasing polarity starting with chloroform. Extraction was carried out at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator and stored in desiccators for further work. The yield of different extracts prepared with chloroform, ethyl acetate, ethanol, methanol and water were found to be 3.45%, 2.12%, 6.79 %, 8.53% and 11.75% respectively.

Phytochemical screening

A preliminary phytochemical screening of all these extracts was carried out [21].

Determination of antimicrobial activity

Microorganisms

For antibacterial activity-*Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*. For antifungal activity-*Aspergillus niger*, *Candida albicans*. These were selected as test microorganisms in the present study.

Culture media

For antibacterial activity- Nutrient agar medium
For antifungal activity-Sabouraud medium
Mediums were procured from HiMedia Chemicals, Mumbai.

Evaluation of antibacterial and antifungal activity [22-25]

Antibacterial and antifungal activity

The *in vitro* antimicrobial activity of different extracts was studied by agar well diffusion method. The antibacterial studies were carried out against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, in nutrient agar medium. Antifungal studies were carried out against *Aspergillus niger*, *Candida albicans* in Sabouraud medium. The medium was sterilized by autoclaving at 120°C (15 lb/in^2). 20ml of sterile culture medium was poured into sterile Petri-dishes and allowed to solidify. The Petri dishes were incubated at 37°C for 24 hours to check for sterility. The medium was seeded with the organisms by pour plate method using sterile top agar (4 ml) contained 1 ml culture. In each plate wells of 8 mm diameter were made using a sterile borer. Bacterial concentration of 1×10^8 CFU/ml was used for antibacterial activity and fungal suspension of 1×10^6 CFU/ml for antifungal activity. The extracts were freshly reconstituted with dimethyl sulphoxide to 2 mg/ml and 4 mg/ml concentrations. The test samples and the control (0.2 ml) were placed in 8 mm diameter well. Antibacterial assay plates were incubated at 37 ± 1°C for 24 h, whereas antifungal assay plates were incubated at 28 ± 1°C 48 h. Dimethyl sulphoxide (DMSO) was used as solvent control (negative control) and maintained at the same experimental conditions. Standard antibiotics ciprofloxacin (0.1 mg/ml) and Fluconazole (0.1 mg/ml) were used as positive antibacterial and antifungal control respectively. Diameter of the zone of inhibition (in mm) surrounding each well was recorded. The extracts that showed significant antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay. All experiments were performed in triplicate.

Determination of MIC values:

The MIC of the extracts was determined using two fold serial microdilution method with saline at a final concentration ranging from 4 mg/ml to 0.0312 mg/ml. The tested extracts were added to sterile Muller- Hinton broth in microtiter plates before the diluted bacterial suspension (final inoculums of 1×10^8 CFU/ml) and (fungal suspension of 1×10^6 CFU/ml) were added. Each extract was assayed in triplicate. The MIC values were taken as the lowest concentration of the extracts in the well of the microtiter plate that showed no turbidity after incubation at 37 ± 1°C for 24 h for bacteria and at 28 ± 1°C 48 h for fungi. The turbidity of wells in the microtiter plates were interpreted as visible growth of microorganisms.

RESULTS:

Pharmacognostic evaluation showed the presence of various chemical components in different extracts. Data is given in **Table 1**

TABLE 1: PHYTOCHEMICAL SCREENING

Chemical component	Chloroform extract	Ethyl acetate extract	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+	+	-
Tannins	-	+	+	+	-
Anthraquinones	-	-	-	-	-
Glycosides	-	+	+	+	-
Carbohydrates	-	-	+	+	-
Saponins	-	+	+	+	+
Flavonoids	-	+	+	+	+
Terpenoids	+	+	+	-	+
Proteins	-	+	+	+	-
Steroids	-	+	-	-	-

(+) Present; (-) Absent

Antimicrobial activity: It is recorded in terms of zone of inhibition in mm. **Table 2** shows value for zone of inhibition for all five extract against all microbial strains.

TABLE *2*: ZONE OF INHIBITION FOR DIFFERENT EXTRACTS

Extract	Concentration (mg/ml)	Zone of inhibition (mm)Mean ± SEM					
		B.s.	S.a.	E.c.	K.p.	A.n.	C.a.
Chloroform	2 mg/ml	08.22± 0.09	09.21± 0.04	08.42± 0.19	08.18± 0.13	09.34± 0.12	10.65± 0.22
	4 mg/ml	09.38± 0.23	10.22± 0.08	09.22± 0.09	08.50± 0.16	11.27± 0.22	11.40± 0.32
Ethyl acetate	2 mg/ml	12.07± 0.07	12.32± 0.09	15.27± 0.32	12.10± 0.09	13.4± 0.11	12.37± 0.15
	4 mg/ml	13.21± 0.15	14.22± 0.12	17.14± 0.22	14.13± 0.12	15.21± 0.14	15.37± 0.21
Ethanol	2 mg/ml	15.24± 0.15	12.57± 0.12	13.12± 0.12	14.37± 0.14	14.17± 0.13	13.15± 0.12
	4 mg/ml	17.21± 0.11	16.23± 0.12	15.13± 0.16	16.23± 0.12	15.24± 0.21	16.27± 0.17
Methanol	2 mg/ml	16.47± 0.14	16.37± 0.06	16.23± 0.08	14.30± 0.21	14.24± 0.15	15.37± 0.14
	4 mg/ml	22.15± 0.24	20.11± 0.10	19.31± 0.09	17.14± 0.16	17.15± 0.11	20.23± 0.12
Aqueous	2 mg/ml	09.15± 0.06	08.36± 0.05	-	-	11.2± 0.09	11.85± 0.17
	4 mg/ml	11.18± 0.09	09.24± 0.10	-	-	13.7± 0.15	11.47± 0.15
Ciprofloxacin	0.1 mg/ml	26.21± 0.11	24.11± 0.12	23.15± 0.24	20.21± 0.08	NA	NA
Fluconazole	0.1 mg/ml	NA	NA	NA	NA	20.16± 0.09	24.21± 0.08
DMSO	-	-	-	-	-	-	-

*Each value is Mean±SEM of 3 assays

NA- not applicable; .B.s.-*Bacillus subtilis*; S.a.-*Staphylococcus aureus*; K.p.-*Klebsiella pneumonia*, E.c.-*Escherichia coli*; A.n.- *Aspergillus niger*; C.a.-*Candida albicans*.

Determination of MIC values of the extracts

TABLE 3: MIC FOR ETHYL ACETATE EXTRACT

Concentration (mg/ml)	Ethyl acetate extract B.s.	S.a.	E.c.	K.p.	A.n.	C.a.
4	-	-	-	-	-	-
2	-	-	-	-	-	-
1	+	+	-	+	+	+
0.5	+	+	+	+	+	+
0.25	+	+	+	+	+	+
0.125	+	+	+	+	+	+
0.0625	+	+	+	+	+	+
0.03125	+	+	+	+	+	+

(+)Presence of growth; (-) Absence of growth

TABLE 4: MIC FOR ETHANOL EXTRACT

Concentration (mg/ml)	Ethanol extract B.s.	S.a.	E.c.	K.p.	A.n.	C.a.
4	-	-	-	-	-	-
2	-	-	-	-	-	-
1	-	-	-	-	-	-
0.5	+	+	+	+	+	+
0.25	+	+	+	+	+	+
0.125	+	+	+	+	+	+
0.0625	+	+	+	+	+	+
0.03125	+	+	+	+	+	+

(+)Presence of growth; (-) Absence of growth

TABLE 5: MIC FOR METHANOL EXTRACT

Concentration (mg/ml)	Methanol extract					
	B.s.	**S.a.**	**E.c.**	**K.p.**	**A.n.**	**C.a.**
4	-	-	-	-	-	-
2	-	-	-	-	-	-
1	-	-	-	-	-	-
0.5	-	-	+	+	+	+
0.25	+	+	+	+	+	+
0.125	+	+	+	+	+	+
0.0625	+	+	+	+	+	+
0.03125	+	+	+	+	+	+

(+) Presence of growth; (-) Absence of growth

DISCUSSIONS: All the extracts showed antimicrobial activity on the some microorganisms under test but ethyl acetate, ethanol and methanol extracts were found to be active against all microorganisms and are more effective, hence subjected to MIC determination. The minimum inhibitory concentration of methanolic extract, ethanolic extract and ethyl acetate extract against most of the test microorganisms were 1 mg/ml, 1 mg/ml, 2 mg/ml respectively. Of the different extracts, the methanol extract displayed the highest antimicrobial activity, as it was evidenced by the highest mean zone of inhibition against maximum of the test microorganism. Preliminary phytochemical screening of the extracts showed presence of flavonoids, glycosides, tannins, alkaloids, terpenoids and polyphenolic compounds.

It has been reported that tannins inhibit many microbial enzymes in raw culture filtrates or in purified forms [26]. The astringent property of the tannins is reported to be due to its complexation with enzymes or substrates and metal ions [27, 28]. Polyphenolic compounds are known to have antimicrobial activity possibly due to enzyme inhibition in the oxidized forms or through more nonspecific interactions with the proteins. Also various secondary metabolites of plant origin are known to possess antimicrobial activity [29].

CONCLUSIONS: The antimicrobial activity found to increases in the extracts in order of ethyl acetate extract, ethanol extract and methanol extract. From the present investigations, we can conclude that asafoetida possesses significant antimicrobial activity due to presence of various phytoconstituents and it could be a source of new antibiotic compounds. The above antimicrobial activity of asafoetida may be due to the combined or individual effect of the present phytoconstituents, which can be further confirmed by the extensive studies.

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