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FORMULATION AND EVALUATION OF ANTIFUNGAL AND ANTIBACTERIAL ACTING HERBAL TABLETS OF AJWAIN

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ABSTRACT The aim of this study is to show ethanolic extract of ajwain has antibacterial and antifungal properties and to formulate tablets of the extract. Trachyspermum ammi commonly known as 'Ajwain' belongs to family 'Apiaceae' is distributed throughout India and is mostly cultivated in Gujarat and Rajasthan. Ajwain seed/fruits were collected from Navi Mumbai and verified by local farmers. These were then triturated in mortar and pestle placed in thimble extracted by soxhlet extraction using ethanol as solvent. Solvent containing the extract was then evaporated until semi solid mass was obtained. It was screened for its antimicrobial and antifungal properties. The extract was used to formulate tablets by wet granulation process. The Antimicrobial and antifungal screening proved that ethanolic extract of ajwain has antimicrobial and antifungal action. It was then combined with various excipient to form granules which were evaluated and placed in single punch compression machine to form tablets. The tablets showed good evaluation parameters.

KEYWORDS : Ajwain, extract, antifungal, tablet.

INTRODUCTION:

Candida albicans and *Staphylococcus* species are, respectively, the most common fungal and bacterial agents isolated from bloodstream infections, worldwide. These species are part of the commensal microbial flora but can cause hospital-acquired infections with an extreme ability to inhabit diverse host niches, especially in immunocompromised patients. They are well known for their ability to form persistent biofilms in the host or on abiotic surfaces such as indwelling medical devices. Interactions within these biofilm communities can lead to increased virulence, drug tolerance, and immune evasion. This can ultimately impact morbidity and infection outcome, often leading to an increased mortality.(*Frontiers | Candida Albicans and Staphylococcus Species: A Threatening Twosome | Microbiology*, n.d.) Along with these *Escherichia coli* is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveller's diarrhoea, and other clinical infections such as neonatal meningitis and pneumonia.(*Escherichia Coli (E Coli) Infections*, 2021) Currently, herbs are applied to the treatment of chronic and acute conditions and various ailments and problems such as cardiovascular disease, prostate problems, depression, inflammation, and to boost the immune system, to name but a few. Herbs and plants can be processed and can be taken in different ways and forms, and they include the whole herb, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract. Plants and herbs extract vary in the solvent used for extraction, temperature, and extraction time, and include alcoholic extracts (tinctures), vinegars (acetic acid extracts), hot water extract (tisanes), long-term boiled extract, usually roots or bark (decoctions), and cold infusion of plants (macerates).(Wachtel-Galor & Benzie, 2011) Extract of Trachyspermum ammi has proven to show action against all three microorganisms.*Trachyspermum ammi* commonly known as 'Ajwain' belonging to family 'Apiaceae' is distributed throughout India and is mostly cultivated in Gujarat and Rajasthan.(Bairwa et al., 2012) In this study herbal tablets were formulated using ethanolic extract of ajwain which showed antimicrobial and antifungal properties.

MATERIAL AND METHODS:

Chemicals:

All chemicals and materials for present study were collected in optimal form from the SRL chemicals and Astron chemicals. All chemicals used for experiment were of analytical grade.

Plant collection and extraction:

Seeds/fruits of *Trachyspermum ammi* were collected from the local shops in Navi-mumbai and identified by local farmers. Dried seeds were triturated in mortar pestle and extracted by using soxhlet extraction process. Ethanol as a solvent was used for extraction. The extraction was carried out for 6 hrs until the solvent in the thimble where the drug was present became colourless.

Antibacterial and antifungal screening:

Bacteria seeded plate: Well method was performed to study anti-bacterial and antifungal activities of essential oil of ajwain were examined against Gram positive (*S. aureus)* and Gram negative bacteria (*E. coli)* and fungi ©. *albicans*). For the determination of zones of inhibition for Gram-positive, Gram-negative and fungal strains were taken along with a standard antibiotic and fungicide for comparison of the results. Control experiments were carried out by using Gentamicin, fluconazole and ceftriaxone as standard drugs. The zones of growth inhibition around the disks were measured after 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivity of the microorganism species to the ajwain ethanol extract were determined by measuring the sizes of inhibitory zones.

Sample Name	Name of Organism		
	E. coli (2.9×10^6 CFU/ml)	*S. aureus* (1.5×10^6 CFU/ml)	*C. Albicans* (2.4×10^6 CFU/ml)
	Zone of inhibition (mm in diameter)		
Ajwain Extract (Ethanol)	20.06	19.92	29.42
Fluconazole Injection USP	30.51	29.24	27.12
Ceftriaxone Injection	40.41	38.36	38.09
Gentamicin	33.90	27.08	24.72

Table no. 1: Antibacterial and antifungal screening

Formulation Of Herbal Tablets:

First the herbal granules were prepared by using wet granulation method. Various batches were prepared using various combinations of binders and then the optimum batch was used with ethanol extract of ajwain seed/fruit. Along with extract as active ingredient lactose was used as diluent and starch was used as disintegrating agent and propylene glycol as binder A damp mass was formed which was passed through sieve no. 10 and then dried in a hot air oven at 70°C for 90 min. Then the dried granules were passed through sieve no .12 and fines were collected through sieve no. 22 .Magnesium stearate and talc were added in formulation with 10% fines. granules were then formed into tablets by using a single punch compression machine.

Table no. 2 : Formulation of tablet by wet granulation

Sr.no	Ingredients	For 1 tablet	For 40 tablet	Activity
1	Ethanol extract	0.125g	5g	Antibacterial and antifungal

2	Lactose	0.375g	15g	Diluent
3	Starch	0.048g	1.92g	Starch
4	Talc	0.04g	1.6g	Glidant
5	Magnesium stearate	0.012g	0.48g	Lubricant
6	Propylene glycol	qs	qs	binder

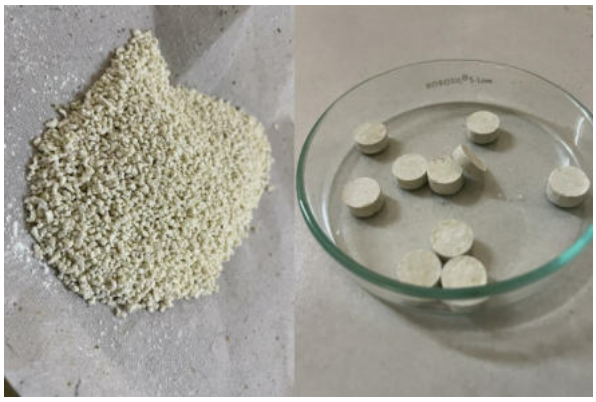

Fig no.1:Formulated tablet and granuels.

Evaluation test of granules:

Angle of repose:

The Angle of repose was tested by the fixed funnel method. The 5 g of powder mixture was poured into a glass funnel. The lower tip of the glass funnel was 5 cm high from the ground. The height (h) and radius (r) of pile were measured, and then calculated as follow:

$\theta = \tan -1h/r$

θ = angle of repose (°)

h = height (cm)

r = radius (cm)…(Pandey et al., 2018)

Bulk density:

The 20 g of powder mixture was weighed accurately, gently poured into a 100 ml glass cylinder without compacting. The volume of powder mixture was recorded, and then calculated as follow:

Bulk density = m/V_0

m = mass (g)

V_0 = unsettled apparent volume (cm^3)…(Pandey et al., 2018)

Tapped density:

The glass cylinder with powder mixture from bulk density testing was used to test tapped density. It was tapped for 25 strokes. The volume of tapped powder mixture was recorded,and then calculated as follow:

Tapped density = M/V_f

m = mass (g)

V_f = final tapped volume (cm^3)…(Pandey et al., 2018)

Carr's index:

Data from bulk density and tapped density testing were used to calculate compressibility index follow Eq

Compressibility index = [(Tapped density – Bulk density)/Tapped density]×100...(Pandey et al., 2018)

Hausner's ratio:

It is a direct indicator of ease of measuring the flow of powder. Hausner ratio was calculated as follow:

Hausner ratio = Vo/V_f

V_0 = unsettled apparent volume (cm3)

V_f = final tapped volume (cm3)…(Pandey et al., 2018)

Evaluation Of Tablets:

Weight variation:

Weight variation 20 tablets were individually accurately weighed. Each tablet weight was recorded. Results were reported .(Pandey et al., 2018)

Friability:

The tablet's dust was removed before testing. 10 tablets were accurately weighed together, and friability was tested using a Roach Friability tester. After 4 min of rotation at 25 rpm, any loose dust from the tablets was removed before accurately weighing again. If friability was not more than 1.0%, it was considered acceptable.(Pandey et al., 2018)

%Friability = (tablet wt before friability - tablet wt after friability/total wt after friability) *100

Hardness:

Tablet requires some amount of strength and resistance to friability to mechanical shock of handling in manufacture, packaging, and shipping. Hardness is thus sometimes termed as the crushing strength. 10 tablets were measured using Monsento hardness tester .(Pandey et al., 2018)

Thickness:

Thickness of the tablet was calculated by Digital Vernier calipers.Tablet was put in between jaws and measured thickness and 6 tablets were used for this test and unit expressed in mm.(Pandey et al., 2018)

DT :

For most tablets, the first important step toward solution is breakdown of the tablet into smaller particles, a process known as disintegration. The DT of the tablet was determined in a phosphate buffer (PH 7.4) at 37 ± 0.5°C using a Disintegration Tester. (Pandey et al., 2018)

EVALUATION OF GRANULES		EVALUATION TEST OF TABLETS	
Tapped density	0.95g/ml	Weight variation	0.42±0.02
Bulk density	0.74g/ml	Friability	0.93%
Carr's index	22%	Hardness	1.5 kg/cm^2
Hausner's ratio	1.28	Disintegration time	50sec
Angle of repose	34.8°	Thickness	4mm

Table no .3 : Evaluation test of granules and tablets.

RESULT:

Antimicrobial screening of ethanolic extract of ajwain showed zone of inhibition of 20.06 mm gram negative bacteria E. coli, 19.92 in gram positive bacteria S. aureus send 29.42 mm in fungal species of C. Albicans. Fluconazole injection USP show zone of inhibition of 30.51 mm in E. coli, 29.24 in S. Aureus and 27.12 in C. Albicans. Ceftriaxone injection showed zone of inhibition of 40.01 mm in E. coli, 38.36 mm in S. aureus and 38.09 mm in C. Albicans . Gentamicin showed 33.90 mm in E.coli,27.08 mm in S. Aureus and 24.72 mm in C. Albicans. Formulated in table no, 1. Granules formed using ethanolic extract of ajwain showed bulk density of 0.74g/ml, tapped density of 0.95g/ml, Carr's index 22%, Hausner's ratio 1.28 and angle of repose of 34.8°. Tablets were then formulated using a single punch compression machine showed thickness of 4mm, Hardness 1.5 kg/cm^2 , Fiability 0.93 % , And disintegration time of 50 sec.

CONCLUSION:

From this study it was concluded that ethanolic extract of Ajwain showed a good zone of inhibition as well as greater zone of inhibition compared to fluconazole and gentamicin in fungal species of C. albicans hence it can be easily concluded that ethanolic extract of ajwain has Antimicrobial and antifungal properties. The granules formulated turned out to be 'passable' after evaluation. Tablets showed good hardness with uniform thickness and less weight variation and fast disintegration time.

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