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PREPARATION OF HERBAL FERMENTED KWATH AND STUDY ITS EFFECT ON NOSOCOMIAL PATHOGENS CAUSING RESPIRATORY TRACT INFECTIONS

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Abstract: Increase in antibiotic resistance and nosocomial infections are problems that have been reported for many years around the world causing economical burden and prolonged treatment. The majority of infections are caused by multidrug-resistant bacteria coming from the hospital-acquired environment. This study aims to eliminate or decrease their antibiotic resistance and to eliminate their Virulence factors, By using traditional medicine in a form of fermented kwatha. As fermentation is a process of preparing formulations wherein therapeutic attributes of a group of ingredients are extracted out of either freshly extracted juice of plants (Asava) or kwatha, a decoction prepared in water (Arishta) with the help of biochemical or microbial fermentation and anaerobic respiration into the liquid. In this study fermented kwath was prepared by using the coarse powder of a plant part in a form of a decoction. Fermentation was carried out for 1 month with decoction and with jaggery and dry dates (kharik) as a source of sugar. The Woodfordia fruticose (Dhataki) flowers and Baker's yeast as fermenting agents. During fermentation self generated alcohol will help to extract phytochemicals from plants which will helps to eliminate virulence factor and will show antimicrobial effect on bacterial isolates. Self generated alcohol also contributes during quicker absorption and preservation. Dry dates shown to give less amount of residual sugar, and will be beneficial for diabetic patient .

Keywords: Nosocomial infections, medicinal plants, Multidrug resistance, fermentation, fermented kwatha (Arishta), antimicrobial effect.

1. INTRODUCTION:

Arishta and Asava are two types of fermentated medicinal preparations in , a traditional system of medicine in India. They are both liquid formulations made by fermenting herbs with water, jaggery or honey, and sometimes other ingredients like fruits or spices. Arishta and Asava are commonly used for treating a wide range of health conditions, including digestive problems, respiratory disorders, fever, and skin diseases. The main difference between asava and arishta is starting step of there preparation. Preparation of arishta start with course powder boiling in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is applied for extracting water-soluble, heat-stable constituents. This process is

typically used in preparation decoction of medicinal plants also called as ayurvedic "kawath" . While asavas are prepared by directly using fresh herbal infusion (fresh juice)(7). Fermentation of both preparations is brought about by the addition of a source fermentation initiator that is *Woodfordia fructicosa* Kurz flowers commonly called as dhataki pushpa or dhaiphool; or flowers of Madhuka (*Madhuka indica* Gmel.). Several studies also reported use of different yeast strains in commercial preparation.(6)

India is home to a vast array of medicinal plants, and has a long history of using plant-based remedies in traditional systems of medicine such as Ayurveda, Siddha, and Unani. In Ayurveda these plants play a significant role in , as they are used for medicinal purposes to treat a variety of ailments. Throughout history, Indian herbal medicinal drug has been used to treat a wide range of illnesses and conditions, including digestive problems, respiratory ailments, skin disorders, and mental health issues. Today, Indian herbal medicine continues to be an important part of the country's healthcare system, As people come more apprehensive of the implicit side effects of conventional drug and the benefits of natural remedies, they're turning to Ayurveda and other traditional systems of drug. In recent times , there has been an increase in scientific research into Ayurvedic medicinal drug , which has helped to validate its effectiveness and safety. Medicinal Plants also have been used for centuries for their antibacterial properties. Many plants contain natural compounds that can inhibit the growth of bacteria and other microorganisms. These compounds called as Phytochemicals, that are naturally occurring compounds found in plants.

Glycosmis pentaphylla is a species of flowering plant in the family Rutaceae. It is a small tree or shrub that can reach a height of up to 10 meters and native in countries such as Thailand, Malaysia, Indonesia, and the Philippines. The leaves are compound, with five leaflets that are oblong to elliptical in shape. It has been traditionally used in Ayurvedic and Siddha medicinal drug for various therapeutic purposes. The plant is used in indigenous medicine for cough, jaundice, inflammation, rheumatism and anaemia.(1).Leaves are considered as good antidote for eczema and other skin troubles and applied in the form of paste. Antioxidative and antimicrobial activity of this medicinal drug have been reported in several studies.(2)

Fumaria indicia, also known as Indian fumitory or Himalayan fumitory, is a plant species in the family Papaveraceae. It is native to the Indian subcontinent and can be found growing wild in many parts of the region. This plant is an annual herb that grows up to 1 meter tall. It have been reported various medicinal purposes to acquire pharmacological activities like antipyretic, Hepatoprotective, Hypoglycaemic, Antidiarrheal, Antispasmodic, Anthelmintic antieczema antiperiodic compound liver complaints and Scrofulous skin affections. Phytochemicals and antimicrobial studies of Fumaria indicia have been reported in serval pervious studies, suggesting it can be versatile candidate for other vedic formulations. (3),(4).

Here this study is aims to use these potential medicinal candidates and to make them available in medicinal formulation to fight against nosocomial infections causing organisms. To make them available in liquid formulation different substrate and fermentation initiator being used.

2 MATERIALS AND METHODS:

2.1 Plant collection and processing of raw material

2.1.1 Fresh leaves of *Glycosmis pentaphylla* commonly called menki in konkani collected from dodamarg village of Sindhudurga district; State: Maharashtra. Leaves were collected in the month of may then washed thoroughly with tap water and dried under shed for 3-4 days. (Till completely dried) and stored In room temperature.

2.1.2 Dried samples of *Fumaria indica*, commonly called Pitpapra or paripatha in Marathi, collected From dodamarg, district Sindhudurga; state Maharashtra ; in may 2022 and stored at room temperature. Both dried samples were grinded into coarse powder using mortal and pestle & stored into airtight container for further use.

2.1.3 Dhataki Pushpa (Flower of *Woodfordia fruticosa*) in dried form purchased from dadar Pharmacy, District Mumbai; state Maharashtra. Dry dates and brown jaggery powder purchased from local market in Mumbai; Maharashtra.

2.2 Microbiological study of flower of Woodfordia fruticosa

2.2.1 Buffered sodium chloride peptone buffered solution was prepared (Mallya Suma V, et al.2019) Potassium dihydrogen phosphate (0.17g), disodium hydrogen phosphate (0.36g), Sodium Chloride (0.21g), peptone (0.05g) was dissolved in 40 ml distilled water and pH was adjusted to 7.0 and the volume was made up to 50 ml. Then buffer solution was autoclaved at 121°C with 15 psi for 15 minutes.(8).

2.2.2 Dried flowers were washed thoroughly with hot water and dried at room temperature. For microbiological analysis 1g of flowers mixed in 10 ml of Buffered Sodium Chloride Peptone solution to make dilution 10^{-1}. 0.1ml of diluted sample was spread onto potato dextrose agar medium and plates were incubated at room temperature for 4 to 5 days.

2.3 **Preparation of kwath or decoction followed by fermentation**

2.3.1 coarse powder added into 800 ml sterile distilled water, and subjected to heat at 90°c to 95°c until it's volume gets reduce to about ¼ (approx.200ml).

2.3.2 Brown jaggery powder and dry dates powder was added as substrate for fermentation. Kwath is cooled at about 60°c to 65°c and then transferred into sterile 150 ml flasks

2.3.3 Fermentation initiator was added and that is dry yeast suspension and dhataki flowers into respective labelled flasks. (According to table no. 1 and table no. 2)

2.3.4 Flasks were plug with cotton then cover with sterile cotton cloth. Kept in dark at room temperature to carry out fermentation reaction.

2.3.5 After month of fermentation samples were filtered through sterile whatman filter paper and stored in sterile flasks for further studies.

2.4 Preliminary Evaluation of arishta Formulations

2.4.1 Organoleptic characteristics odour, taste, colour and clarity of prepared arishta formulation was determined.

2.4.2 **pH**: Digital pH meter was used to check the pH of the broth. The pH meter was calibrated by using standard buffer solution of pH 4.0, 7.0 and 9.2.

2.4.3 **Determination of ethanol content by dichromate oxidation method followed By redox titration**.

Quantitatively pipette out diluted sample into another flask. Stand flask in large plastic tray of ice & water. Using a volumetric pipette, very slowly 20.0mL of the Potassium Dichromate solution was added drop wise into the flask (There will be generation of heat in the starting of reaction, so it is important that reaction mixture remains cold during the addition of the dichromate solution ,to prevent the loss of acetaldehyde vapour.). Flask was placed in a water bath at 60-65°c. Leave sample to react for at least 30 minutes.(at this point temperature should not be more than 65°c) Blank was prepared with distilled water and 20.0mL dichromate solution. Both the flasks were kept in water bath. The sample and blank flasks were removed and allow to cool. Back titration was performed quantitatively by transferring the contents of the sample and blank into another 250ml flask. Burette was filled with ferrous ammonium sulphate solution. Blank titration was performed using the prepared blank sample. Titrated with ferrous ammonium sulphate until the solution turned to an emerald green. 5 drops of Phenanthroline ferrous sulphate indicator was added to the solution. Titration was continue until the colour changed from emerald green- blue to brown. Sample titration was performed same as the blank titration. Blank titre VB was recorded. Sample titre VA was recorded. Ethanol content was calculated from formula.

2.4.4 Estimation of reducing sugar by DNSA method

0.5mg/ml -3mg/ml standard glucose solution were prepared. 3ml of sample and 3 ml of standard solution was taken into test tubes and equal amount of DNSA reagent was added. Reaction mixture was heated in boiling water bath for 5 mins and cooled at room temperature. Absorbance was measured at 530 nm. Amount of reducing sugar present in the sample was calculated using the standard glucose curve. Percentage of reducing sugar calculated.

2.4.5 Preliminary phytochemical screening of fermented kwath samples.

A. Test for Flavonoids
   - 5ml dilute ammonia is added to 2ml of sample with few drops of concentrate H_2SO_4 Intense yellow color indicates positive results for test.
   - 2ml sample was shaken with 1Nsodium hydroxide Solution , yellow color indicated positive test for flavonoids which becomes colourless after addition of 1%HCl .

B. Test for tannins & phenols
   - 2ml of sample with 1%ferric chloride solution. A blue- green , blue-black colouration indicated presence of phenols and tannins.
   - Sodium hydroxide test:5 ml of sample was dissolved in 0.5ml of 20% sulphuric acid solution. Followed By addition of few drops of aqueous sodium hydroxide solution, it turned blue which indicated the presence of phenols.

C. Test for Saponins
   - Foam test 2ml of sample mixed with 5 ml of distilled water and few drops of olive oil and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

D. Test for steroid
   - Salkowski's test : 5ml of sample mixed with 2 ml of chloroform and 3 ml of concentrate H_2SO_4 added carefully to form a layer. A reddish brown colour ring indicated the presence of steroidal ring.

E. Test for alkaloids
   - Wagner's test: 1 ml of sample is mixed with 1ml wager's reagent (potassium iodide) and the formation of a reddish brown precipitate indicated the presence of alkaloids.
   - Dragendorff's test:1ml of sample mixed with , a few drops of Dragendorff's reagent. Appearance of orange colour indicates the presence of alkaloids.

F. Tests for Glycosides

   - Keller-kilani test:2ml sample was mixed with 2ml of glacial acetic acid Containing 1-2 drops of 2% Solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.
   - Liebermann's test :2ml of sample mixed with each of 2ml of chloroform cooled 2ml of acetic acid. The mixture was cooled then concentrate H_2SO_4 is added. And the colour bluish green indicated the presence of glycosides.

2.5 Antimicrobial efficiency testing of fermented kwath

2.5.1 preparation of test micro-organism

The organisms: *Serratia marcescens, Klebsiella .pneumoniae* obtained from kooper hospital Mumbai. Test organisms suspension was prepared by inoculating it into sterile Luria bertanii broth and incubated at 37°c for 24 hrs. *Serratia. marcescens* inoculated in LB broth and incubated at room temperature for 24 hrs. Then the turbidity of the microbial suspension was adjusted by McFarland standard OD of 0.5 at 600 nm.

2.5.2 Agar well diffusion method was also performed to get antimicrobial effect of fermented kwath. 0.1ml of prepared test inoculum were inoculated into MH medium. Fermented kwath samples were added into the 6mm agar well. *Serratia marcescens* plates incubated at room temperature and *Klebsiella pneumoniae* plates were incubated at 37°c for 24hrs to obtained zone of inhibition. Zone of inhibition showed by of kwath compared with zones given by standard antibiotic. Positive Control Ofloxacin (5mcg) was used for *Serratia marcescens*, and Ciprofloxacin (5mcg) for *Klebsiella pneumoniae*.

3 RESULTS

3.1 Microbiological study of *Woodfordia fruticosa* flowers

10^{-1} diluted suspension of dry flowers spread onto Potatoes dextrose agar medium, after incubation fungal colonies observed for their microscopic structure. Part of growing fungal colony were placed on slide with drop of crystal violet stain, oval shaped cells with psuedohyphal morphology of fungal flora observed under 100x magnification.

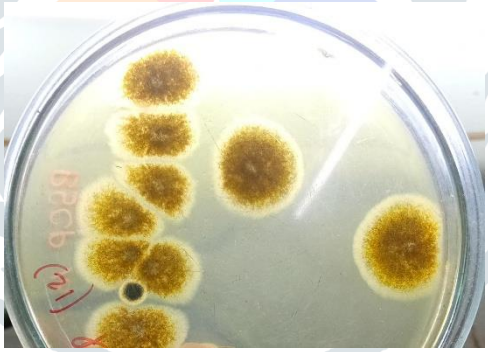

Fig. 1 colonies of microbial flora from *Woodfordia fruticosa* flowers

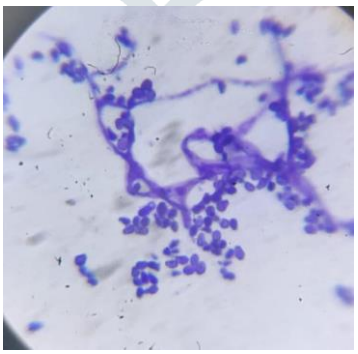

Fig. 2 morphology under 100x magnification

Table no.1 samples labelled according to substrate and fermentation agent

Fig no. 3 prepared fermented kwath for sample A *Glycosmis pentaphylla*

Sample A G.pentaphylla	**Substrate**	**Fermentation agent**
Flask1(AD1)	Dry date powder	Yeast suspension
Flask2(AD2)	Dry date powder	Dhataki flowers
Flask 3 (AJ1)	Brown jaggery powder	Yeast suspension
Flask4(AJ2)	Brown jaggery powder	Dhataki flowers

Table no. 2 samples labelled according to substrate and fermentation agent

Fig no 4 prepared fermented kwath for sample B *Fumaria Indica*

Sample B F.Indica	Substrate	Fermentation agent
Flask 1(BD1)	Dry date powder	Yeast suspension
Flask2(BD2)	Dry date powder	Dhataki flowers
Flask 3(BJ1)	Brown jaggery powder	Yeast suspension
Flask 4 (BJ2)	Brown jaggery powder	Dhataki flowers

3.2 Preliminary Evaluation of arishta Formulations

Table no. 3 fermented kwath of sample A *Glycosmis pentaphylla*

Sample A	**Rupa (colour)**	**Gandha (odour)**	**pH**	**%reducing Sugar**	**% alcohol content**
Flask 1(AD1)	Greenish brown	Slightly alcoholic	4.37	7.3%	7%
Flask2(AD2)	Brownish green	Alcoholic	3.56	5.6%	12.4%
Flask3 (AJ1)	Greenish brown	Slightly alcoholic	4.24	7%	9.2%
Flask4 (AJ2)	Greenish brown	Alcoholic	3.4	6%	12%

Table no. 4 fermented kwath of sample B *Fumaria Indica*

Sample B	**Rupa (colour)**	**Gandha (odour)**	**pH**	**%reducing sugar**	**%Alcohol content**
Flask 1(BD1)	Dark brown	Slightly alcoholic	4.87	7.3%	8.25%
Flask2(BD2)	Dark reddish brown	Alcoholic	3.55	6.2%	10.9%
Flask3 (BJ1)	Dark Brown	Slightly alcoholic	4.06	10.3%	8.6%
Flask4 (BJ2)	Dark brown	Alcoholic	3.72	9.1%	10.5%

3.3 phytochemical screening of fermented kwath samples.

Phytochemicals analysis showed presences of expected phytochemicals in fermented kwath of both the samples.

3.4. Antimicrobial effectiveness of fermented kwath

Table. No 5 Antimicrobial action of Fermented kwath on nosocomial pathogens

Fermented kwath samples	***S.marcescens***	**A.I.**	**P.A.**	***K. pneumoniae***	**A. I**	**P. A**
AJ1	0	0	-	0	0	-
AJ2	0	0	-	7mm	0.23	23%
AD1	0	0	-	6.5mm	0.21	21%
AD2	11 mm	0.45	45%	15mm	0.5	50%
BJ1	0	0	-	8mm	0.26	26%
BJ2	16mm	0.66	66%	17mm	0.56	56%
BD1	0	0	-	0	0	-
BD2	8.5mm	0.35	35%	16mm	0.53	53%
Positive control	24mm			30mm		

mm : millimetre ;A.I : Activity index ; P. A. : percentage activity

Fig.no 5 Antimicrobial effectiveness of fermented kwath on *Serratia marcescens*

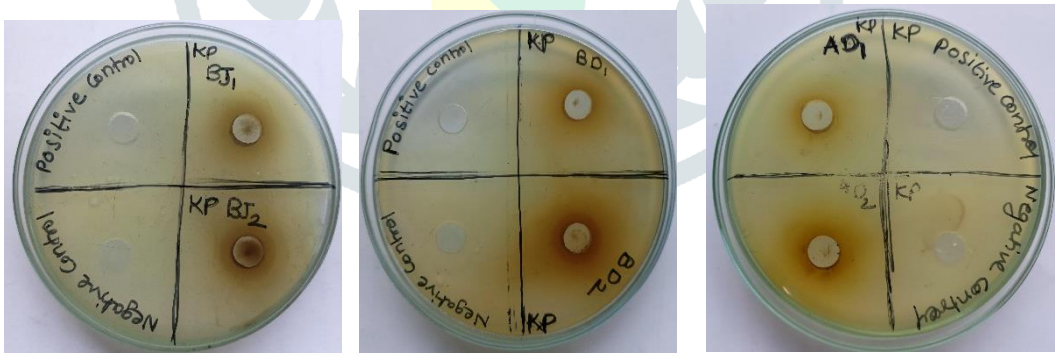

Fig. no 6 Antimicrobial effectiveness of fermented kwath on *Klebsiella pneumoniae*

4 DISCUSSION:

Nosocomial respiratory infections are infections that occur in patients who are receiving medical care in a hospital or other healthcare facility. These infections can be particularly dangerous for patients who are already ill, as they can cause serious complications and even death. The inappropriate use of antibiotics, such as prescribing them for viral infections, or using them for longer than necessary, can lead to the development of resistant strains of bacteria. As the prevalence of antimicrobial resistance increases in healthcare settings, it becomes more difficult to treat infections with antibiotics.

Gram-negative bacteria are an important class of microbial agents for respiratory infections. Klebsiella pneumoniae is known for its ability to develop resistance to antibiotics, This resistance can make it difficult

to treat infections caused by these bacteria. Serratia marcescens is an another important nosocomial pathogen and the depicting characteristic property of antimicrobial resistance. Serratia marcescens is usually shows resistant to antibiotics by producing deoxyribonuclease (DNase), lipase, gelatinase and ability to produce red-pigmented prodigiosin. Therefore, it is essential to promote our traditional system of ayurveda to fight against antimicrobial resistance.

Arishta and Asava are one such Ayurvedic preparations that involve fermentation of herbs and other natural ingredients with the help of self-generated alcohol. Also preparation these fermented kwath with different sugar source another than jaggery or honey can overcome the problem for prescribing it to diabetic patients. As diabetes is becoming one of main interfering factor in treatment of many infection.

In this study two different indigenous herbal plant having background of their use in traditional practices were used to prepare fermented kwath by tradition as well as modified method. Dry leaves of *Glycosmis pentaphylla* and dry stem and seeds of *Fumaria Indica* was used for preparation of fermented kwath (arishta). Four different samples of each plant material was prepared by using different fermenting agent mainly, dry *woodfordia fruticosa* (dhataki) flowers and yeast, also different substrate for fermentation mainly, brown jaggery powder and dry dates (Kharik). Samples were analysed after one month by employing physiochemical parameters and for antimicrobial activity against nosocomial pathogens. Significant variations was observed when comparison was done taking fermenting agents as variable. The results reveals that more amount of alcohol formed in fermentation of kwath with dhataki flowers as compare to with yeast as a fermentation agent. An attempt was also made to find out the effect of different sweetening agents in fermentation process for amount of reducing sugar. Results highlighted that, amount of reducing sugar found to be higher in formulation with brown jaggery powder as substrate than formulation with dry dates. Also suggested that, microbial flora of flowers was able to utilize dry dates as substrate and production of alcohol. According to results of antimicrobial studies, fermented kwath prepared from *Glycosmis pentaphylla* and *Fumaria indica* has shown significant effect on growth of test pathogens. Fermented kwath of *Fumaria Indica* showed more significant antimicrobial activity against both test Pathogens as compared *Glycosmis pentaphylla*. Kwath samples fermented using dhataki flowers was found to be more effective to inhibit growth of test pathogens. However, kwath samples prepared by using commercial yeast could not inhibit the growth of *S.marcescens* and found to be less effective to inhibit the growth of *K. Pneumoniae* as compared to other effective samples. This results also suggesting, microbes associated with dry woodfordia fruticosa flowers involved in fermentation process and mediating this process; enhanced therapeutic properties of plant. Thus, this study was concluded , *Glycosmis pentaphylla* and *Fumaria indica* can be a promising source of natural antimicrobial agents on nosocomial pathogens. They can be maid available in easy administrative form by formulating them in as arishta. Dry dates can be use as good alternative for traditional substrates. And At the meantime, diabetic patients can also adopt ayurvedic treatments. However, Alcohol content should be control and maintain, and it should not be more than 11%v/v as per traditional suggestions.

5 AKNOWLEDGEMENT:

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