

Role of Fermentation methodologies in Traditional medicines

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ABSTRACT

*Fermentation of these herbs can be an important procedure to improve the therapeutic properties of the herbs by modulating both the quality and quantity of different phytochemicals. The fermentation process causes the decomposition of the cells of the herbs resulting in better leaching of active phytochemicals, increasing the concentration of phytochemicals and also conversion of the metabolites into newer biologically active compounds. Fermentation technique has always been an important aspect in different traditional system of medicine be it in Ayurvedic system for the formulation of Arishta and Asava, in Afrikan system of medicine, in Unani system where fermentation is carried out using *Mycoderma aceti*, in Siddha system for the preparation of rice vinegar or Annakaadi, or in Chinese system of medicine where fermentation is a very conventional procedure to improve the therapeutic quality of herbs. In this review, information and details have been collected from various sources and examples are demonstrated how fermentation process resulted in the production of various Phytochemicals. A table has also been established illustrating the various herbal formulations along with their fermenting agent and their respective therapeutic effects.*

Keywords: *Ethnomedicine, Solid state fermentation, Ayurveda, Unani, Siddha.*

1. Introduction

Since ancient times, humans have been dependent on the resources of Nature for their basic needs of food, shelter, clothing, tools and equipments, fertilizers, flavouring agents and, not the least, medicines. The fact that during the ancient times there was not sufficient information related to the illness or about the medicinal properties of the plants proved that everything was based on experience [1]. The drug discovery process of natural products can be elaborated as the following steps, first is the selection of sources that can be plants in which specific secondary metabolite of plants like Alkaloids, Glycosides, Flavonoids etc can be sought, an information-based approach can be done where selection is done based on database surveillance, and finally selection can be done based on Ethnomedical approach by investigating the organism's medicinal use in local areas [2]. The second step is to prepare initial extracts and undergo preliminary biological screening, generally terrestrial plants are extracted with polar solvents like Methanol and Ethanol and then they are partitioned with non-polar solvents like hexane and petroleum ether. Marine or aquatic plants are usually extracted with a mixture of methanol and dichloromethane. Then these extracted samples undergo "High Throughput Screening" (HTS) assays to identify the lead molecule. In such assays, a large number of extracts are evaluated in cell and non-cell-based formats [3]. The third step is to elucidate the lead compound structure and identify them; this involves Bioassay- directed fractionation that isolates the pure active constituent from another biomass. A variety of chromatographic separation techniques like Column chromatography and High-performance liquid chromatography are implemented for this process. The structure elucidation of the pure isolated constituent is done using highly advanced Nuclear Magnetic Resonance and Mass Spectroscopy [4].

2. General Fermentation Methodologies and Conditions

Fermentation process can be defined as the transformation of low-quality substrates into a more enhanced quality product under the influence of microorganisms. Herbal medicines include all the materials that contains the active therapeutic ingredient, these can be leaves, flowers, fruits, seeds, stems, wood, bark, roots, rhizomes, gums, fixed oils, essential oils, resins etc. All of these components can be fermented to increase the composition and properties of the essential phytochemicals present in them [5]. Evidence based researches have been performed to demonstrate that for some herbal therapeutics to perform well or make them more active therapeutically, they need to be converted into more stable form and that occurs due to the beneficial probiotics present in the intestines of the body. Because of such emerging evidences, fermentation can be considered to be a key process for enriching the therapeutic effect of these herbal medicines. Now the mechanism of microbial fermentation of these medicines and their biotransformation can be elaborated in to the following ways. (1) The secondary metabolites formed during the biotransformation of the herbal medicines can also form new compounds under the influence of the microorganisms responsible for fermentation. (2) Further new therapeutic compounds can also be produced by the alteration of the common Biochemical pathways of the microorganism. (3) The therapeutic components of the herbs can be concentrated as the microorganism can consume and digest the non-therapeutic components like proteins, sugars and other substances [6]. On the basis of substrate involved to be fermented, the fermentation techniques can be elaborately divided into two categories: "Solid State Fermentation" (SSF) and "Sub-merged Fermentation" (SmF)/ "Liquid Fermentation" (LF). Now in the process of "Solid State Fermentation" the fermentation is usually done in the complete absence of any kind of moisture, that is the microorganisms cultivates on anhydrous state of substrates. This type of fermentation is most suitable when the fermented microorganisms which are employed to facilitate the fermentation are classes of bacteria, fungi and yeast as they can grow and cultivate in the relative absence of moisture [7]. "Submerged Fermentation"/ "Liquid fermentation" (SmF/LF) involves the microbial growth in aqueous environment of the substrate medium. As a result, this type of fermentation is beneficial for the growth of microorganism like bacteria and some specific fungi that requires high moisture for survival. However, because of the strict maintenance needed for this technique restricts this technique as compared to SSF [8]. The conditions for fermentation need to be optimized for obtaining the desired products, fermentation occurred in unoptimized conditions may result in low quality products. Few of the fermentation conditions are

discussed as follows; (1) Nature of microorganism employed for the process. This suggested that the fermentation capability to digest carbohydrates was greater for *Bacillus subtilis* than other bacterial strains [9]. (2) pH is considered another important parameter influencing the herbal fermentation. Studies have revealed that the anti-oxidant effect of the herbs depends on the pH of the fermentation medium, where the pH can modulate changes in the Polyphenolic compounds thereby altering the anti-oxidant effects [10]. (3) Temperature also plays crucial role in regulating the process of controlled fermentation, this is because by attending a proper optimum temperature will result in increased microbial growth and enzymatic activity. Studies have been conducted to evaluate the effect of temperature in microbial fermentation, where the "*Panax notoginseng*" is evaluated of its hepatoprotective effects against carcinoma. It is determined on Hep3B lines in different temperatures like 32, 37 and 42°C. The result showed that the best anti-tumour activity was shown during fermentation at 37°C, indicating that at 37°C fermentation of *P. notoginseng* gave rise to increased anti-hepatotumour bioactive compounds [11].

3. Fermented Methodologies in traditional system of Medicines

The practice of herbal medicines being fermented prevail in many traditional systems of medicines, those methodologies are discussed as follows:

3.1 African system of medicines

African traditional medicines are considered to be one of the oldest and one of the diverse of all the traditional system of medicines. Well-known medicinal plants of Africa include *Acacia senegal* (Gum Arabic), *Agathosma betulina* (Buchu), *Aloe vera* (North African Origin), *Artemisia afra* (African wormwood), *Boswellia sacra* (Frankincense), *Commiphora myrrha* (Myrrh), *Harpagophytum procumbens* (Devil's claw), *Parkia biglobosa* (African Locust bean) etc [12]. *P. biglobosa* have been used for local consumption as well as for medicinal purposes in the Afrikan continent for ages. They were usually fermented to increase its major nutritive and therapeutic effects. Later a scientific research has been conducted to compare the nutritional properties of raw and fermented locust beans. For fermenting the locust beans were soaked in water for 7 days, then they were dehulled and rinsed and cleaned with water. After that these beans are dried in an oven at 60°C, followed by dry milling and sieving. These beans were assayed for their nutritional properties. The result showed that the fermented locust beans have higher ranges of protein content and energy values than the raw locust beans. Comparative studies also proved fermented beans showed increase in the amino acid profiles of Arginine and Histidine which are very important for the growth and development of growing infants [13].

3.2 Ayurvedic system of medicines

Fermentation is a prescribed method of preparing herbal medicines like Asava and Arishta in Ayurveda. The formulation process of Asava and Arishta only differs in the incorporation of the herbal drug, where the former is used as powder in water, while the latter is used in the form of decoction. In both of these solutions sugar with powdered *Woodfordia fruticosa* flowers are added. The fermentation vessels are then closed shut and kept in warm moist place for about a month to finally yield Asava and Arishta respectively. Instead of *Woodfordia fruticosa* flowers, brewer's yeast (*Saccharomyces cerevisiae*) can also be used to make the fermentation faster within a week. The flow diagram is depicted in Figure 01. The yeast culture which is present on the *W. fruticosa* flowers are the prime microorganisms used for the fermentation of the ayurvedic herbs [14]. In ancient Ayurveda, the exact term "Solid State Fermentation" was never used; however, a crude form of this process was employed. For example, "*Bilwadi Gutika*" (pills) was prepared by triturating the fine herbal powder and then mixing the triturated herbal powder with goat's urine and after that small handmade herbal tablets were prepared. These pills were used for the treatment and amelioration of painful bites like bites from poisonous

scorpions, snakes, spiders and other insects and also for other ailments like gastroenteritis, dyspepsia etc [15]. Another fermented product is “*Kanji*” which is found to be very nourishing and helps in relieving burning sensation and relieves thirsts [16].

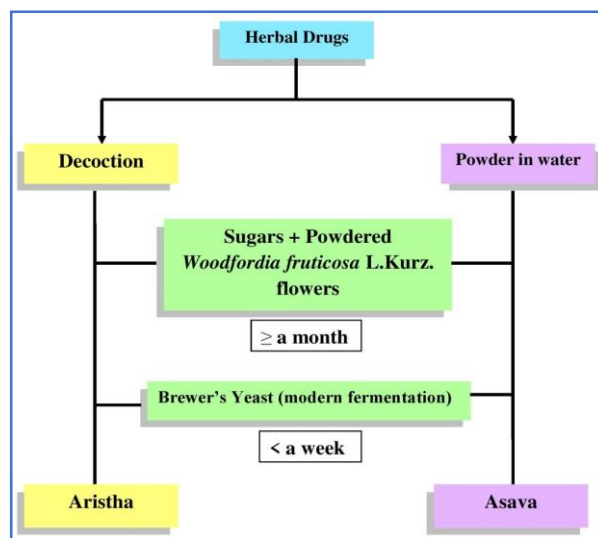


Figure 01: Preparation of Arishta and Asava, based on Ayurvedic system of medicine

3.3 Unani system of medicines

The Unani system of medicine is considered to be one of the traditional systems of medicine practiced in India since the last century. This system of medicine is based on the extraction of bioactive constituents from different herbs. Different types of extraction processes like Mashing, maceration, fermentation, solid phase extraction is employed. Fermentation of the herbal drugs can be considered to be the process of bio transforming the initial herbs to another compound of high therapeutic interest by the application of microorganisms. This process occurs in various ways by rupturing the plant cell wall with the enzymes produced by the microorganisms thus allowing higher amount of bioactive contents to be leached out in the menstruum, or the microorganism can use the herbs as substrate to produce new therapeutically active entities [17]. *Takhmīr* is the English equivalent of fermentation. In Unani system of medicines, the process of fermentation has been employed in the formulation of drugs like *Sirka*, *Nabeez*, *Dar Bahra*, *Aabkaama* etc. *Sirka* is another formulation is prepared by the process of fermentation by microorganism *Mycoderma aceti* or *Ummul Khal* (Mother of vinegar). This formulation is used as anti-hypertensive, anti-infective, cardioprotective, anti-tumour, anti-oxidant etc purposes. *Aabkaama* is also a liquid preparation made up from *Rae* utilizing the same procedure as *Sirka*. *Nabeez* is a unique dosage form that was used in the ancient Unani tradition; it was prepared by the microbial fermentation of aqueous solutions of different grains, fruits, and other medicinal herbs [18]. *Dar Bahra* was prepared by fermenting the cold infusion of herbal drug with sugar, jiggery, and honey. *Sikanjabīn* is another dosage form that would be prescribed in the Unani system of medicine and was prepared with vinegar and honey but later honey was substituted with sugar [19].

3.4 Siddha system of medicines

Another traditional system of medicine is the Siddha system which was founded by the traditional Siddhars of India and this traditional system is established on Dravidian (Tamilian) culture. This combination of medicine is composed of many specialties like “*Annakaadi*” or rice vinegar, “*Jayaneer*” which is a salty extraction, “*Amuri*” which the specialized Siddha elixir, and “*Theeneer*” which are the fermented distillates [20]. “*Annakaadi*” is considered as one such important siddha resource prepared by fermenting rice water. A highly extensive procedure is followed for the preparation of *Annakaadi*. About 325 gm amount of “*Karunkuruvai*” rice is boiled and cooked for about 20 mins inside a big earthen pot. This cooked rice along with its slurry gruel is poured in another pot and to it about 7800 ml of fresh cold water is added. Then on the mouth of the earthen pot a clean white muslin cloth is tied and is covered shut. Then the pot is placed under the sun for “*Suriya pudam*”. The earthen pot is replaced every week. Soon after 30 days it is observed that the boiled rice dissolves, so 325 gm of boiled rice is added again. The process is repeated for several months with periodic changers in pot and addition of boiled rice. After 2 to 3 months of fermentation the “*Annakaadi*” or rice vinegar is ready for administration [21]. The flow diagram of preparation is depicted in Figure 02. This “*Annakaadi*” preparation can now be used for various purposes like; (1) It can be used as an ingredient for Purification or detoxification of raw materials commonly employed in the siddha system of medicine. The process of detoxification of any poisonous drug before it is administered to remove any toxic effects and thereby increasing the efficacy is termed as “*Suthi murai*” (purification process). This following procedure is being employed on various potent drugs having narrow range of therapeutic activity, obtained from herbal, animal or mineral source to reduce its “*Kutram*” (different impurities, low quality substrates or toxic substituents) [22]. (2) “*Annakaadi*” is also used as an ingredient for formulating higher orders of drugs to make the formulations more effective, increase its shelf life, making the minimal dose effective and medication period short [23]; (3) As a medicinal vehicle (*Anubhanam*) for administration of siddha drugs, “*Annakaadi*” has been used for the administration of siddha drugs like “*Sanjeevi Mathirai*”, “*Naga parpam*”, “*Kantha papam*”, “*Thalaga parpam*”, thereby increasing their therapeutic efficacy and potency [24].

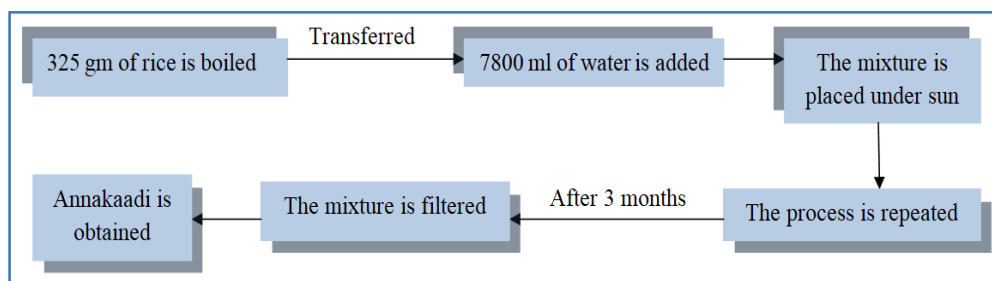


Figure 02: Preparation of *Annakaadi*, based on Siddha system of medicine

3.5 Chinese system of medicines

Chinese system of medicine is considered to be more than 5000 years old, and is based upon theories about the natural laws that maintain the good health and longevity [25]. The fermentation processes are usually conducted employing Solid state fermentation (SSF). In SSF, the fermentation is conducted in low moisture content (approximately 12%) with reduced need for energy and cost requirements [26]. The fermentation process of Chinese medicinal herbs can lead to various advantages, (1) Reduced toxicity, various herbal components when administered in the body can act as foreign material in the body thus producing gut disturbances, allergies, irritation. Therefore, fermentation of those herbs is a good way to reduce the toxicity. Fermentation not only protects the active therapeutic component but also structurally modifies the toxic compounds to render them toxic less in the body. For instance, *Rhei radix et Rhizoma* contains unwanted diarrhea producing anthraquinone derivatives, however on fermentation there is a considerable decrease in the anthraquinone compounds while the content of free anthraquinone with anti-tumour effect is increased six fold [27]; (2) Increased efficacy, the Chinese herbs are generally dense with tough cell wall thus the bioavailability and the potency of the active

pharmaceutical ingredient becomes very low when administered orally. Furthermore, the molecular masses of the herbs are generally quite higher than 500 daltons, making them almost impenetrable towards the movement through blood brain barrier. However, upon controlled fermentation of these herbs, the extracellular catalyzing enzymes of microorganism like Cellulases, Amylases, Amidases and Pectinases degrade the tough cell wall making the cellular components to get extracted out and release the potent therapeutic compounds. For instance, the SSF of Chinese herb *Salvia miltiorrhiza* resulted in increased extraction of phenol, with increase in antibacterial and antioxidant effects [28]. (3) Production of new therapeutic compounds, during the fermentation process, the primary and secondary microbial metabolites can react with the active component present in the herbs to produce a separately new entity. For instance, when *Panax notoginseng* is fermented with *Bacillus subtilis*, Ginsenoside RH4 was isolated from the fermented product which was not present earlier in the pre-fermented compound [29,30].

4. Fermentation of some common Herbal Formulations

Different traditional herbs are fermented with the help of different microorganisms, thus revealing potent health benefits, some of them are represented in Table 01.

Table 01: Tabular representation of some common herbal formulations and their health benefits

Sl. No.	Herbal Formulation	Scientific name	Micro-organism used	Therapeutic Effect	Reference
1	Ashwagandha	<i>Withania somnifera</i> (Solanaceae)	<i>Saccharomyces cerevisiae</i>	Anti-inflammatory effect, Anti-oxidant effect, balances the nerves and adrenals.	[20-23]
2	Birch	<i>Betula pendula</i> (Betulaceae)	<i>Lactobacillus reuteri</i>	Balances the gut micro-flora, anti-microbial and anti-oxidant effects.	
3	Cinnamon	<i>Cinnamomun cassia</i> (Lauraceae)	<i>Lactobacillus plantarum</i>	Controls inflammatory reactions, boosts immune system, overall body detoxification	
4	Dandelion	<i>Taraxacum officinale</i> (Asteraceae)	<i>Lactobacillus plantarum</i> & <i>Saccharomyces cerevisiae</i> in a ratio of 3:7	Anti-oxidant properties, maintains a healthy intestinal tract.	
5	Echinacea	<i>Echinacea purpurea</i> (Asteraceae)	<i>Lactobacillus plantarum</i>	Antimicrobial activity towards Gram-positive and -negative bacteria, Antioxidant effects	
6	Fennel	<i>Foeniculum vulgare</i> (Apiaceae)	<i>Lactobacillus acidophilus</i>	Overall body detoxification, boosts lymphatic system, eliminate toxins and fluid retention, good for urinary tract infection.	[24,25]
7	Garlic	<i>Allium sativum</i> (Alliaceae)	<i>Saccharomyces cerevisiae</i>	Anti-cancer effect through inhibiting cell proliferation, Dyslipidemia and anti-obesity effect	
8	Ginger	<i>Zingiber officinalis</i> (Zingiberaceae)	<i>Lactobacillus plantarum</i>	Overall body detoxification, boosts immune system, maintains cardiovascular system, anti-ulcer, anti-	

				constipation,carminative.	
9	Holy basil	<i>Ocimum tenuiflorum</i> (Lamiaceae)	<i>Lactobacillus acidophilus</i>	Helps in relieving stress, boosts immune system and digestive system, anti-oxidant properties	
10	Turmeric	<i>Curcuma longa</i> (Zingiberaceae)	<i>Lactobacillus plantarum</i>	Anti-cancer, hepatoprotective and neuroprotective. Prevented the cell death induced by oxidative stress in C6 cells. Inhibited the production NO and PGE ₂	
11	Tea	<i>Camellia sinensis</i> (Theaceae)	<i>Eurotium cristatum</i>	Anti-bacterial activity	
12	Soybean	<i>Glycine max</i> (Leguminosae)	<i>Folium mori</i>	Relieve post-menopausal symptoms and reduce the risk of osteoporosis in women	
13	Amla	<i>Embllica officinalis</i> (Phyllanthaceae)	<i>Beauveria bassiana</i>	Anti-angiogenic-like properties	
14	Red ginseng	<i>Panax ginseng</i> (Araliaceae)	<i>Lactobacillus fermentum</i>	Prevents allergic rhinitis results from specific IgE-mediated allergic reactions in the nasal mucosa.	[26-30]
15	Radix astragali	<i>Astragalus membranaceus</i> (Fabaceae)	<i>Bacillus subtilis</i>	Inflammatory, antioxidant, and immunomodulatory functions, great potential for reducing uric acid.	

5. Conclusion

For thousands of years herbal drugs are being employed as traditional medicines for their various therapeutic activities such as immunomodulatory effect by modulation of the immune cells, anti-inflammatory effect by suppressing the inflammatory mediators, anti-diabetic effects by decreasing the level of blood sugar, anti-microbial and anti-infective effects towards microorganism, and anti-allergic effects by mast cell stabilization. Ethnomedicinal knowledge and experience described how fermentation technique can be considered to be an indispensable and vital traditional preparative procedure employed for the enhancing and determining the efficacy and on the other hand reducing different harmful effects of herbs. Later scientifically proved evidences demonstrated how fermentation causes decomposition of the plants cell thereby extracting essential bioactive metabolites and also biotransformation of complex substrates into simple compatible components, thereby modulating the quality and quantity of certain bioactive compounds. In modern era, researchers and scientists are now trying to improve the methods of fermentation process with stable and standard conditions, with newer strains of microbes as well as other technologies to improve the potential of fermentation technologies across the globe. Thus, the quality of herbal drugs can be further be enriched through production of more additional bioactive metabolites including alkaloids, isoflavones, Saponins, Phytosterols, Phenolic compounds, Glycosides, Terpenoids with amelioration of other toxic and unwanted components. However, maintaining the recreatability of the fermentation procedures and also sustaining the optimal purity of the different isolated therapeutic compounds obtained from the fermentation process are some of the main obstacles in such procedures. Fortunately, as the modern era approaches with more sophisticated technologies and enhanced fermentation techniques, these obstacles can be overcome with more prosperous approach in order to enhance and further revamp this potential.

6. Future Aspects

Fermentation has been a predominant approach for enriching and enhancing the herbal medicines in many traditional systems of medicines, be it in the ancient Ayurveda for the preparation of Asava and Arishta, in Afrikan system of medicine, in Unani system where fermentation is carried out using *Mycoderma aceti*, in Siddha system for the preparation of rice vinegar or Annakaadi, or in Chinese system of medicine where fermentation is a very conventional procedure. However, in those days this practice was not scientifically proven and it remained as a hypothetical practice being passed down for several generations. In today's era with more scientific advancements and sophisticated technologies it has been much easier to scientifically prove this approach. Different chemical tests of phytochemicals, different isolation procedures like High performance Liquid Chromatography, Mass Spectroscopy, Nuclear Magnetic Resonance and different clinical research on cell lines or tissues has proved that Fermentation is indeed an excellent strategy for enriching the bioactive chemicals present in the herbal drugs. As the effectiveness of fermentation continues to revolve around the globe, more researchers are trying to reorient this technique in to an essential step of formulation. Several industries are utilizing fermentation techniques on large scale like in brewers and stainless-steel silos to make more enhanced and supplemented herbal drugs. Fermentation techniques in the upcoming modern era can be a huge aspect and can completely re-orient and incorporate the traditional system of medicine in modern groups of therapeutics, thus having amplified therapeutic effects with better patient compliance and lower side effects.

7. References

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