Chapter 1

Introduction

1.1.Food as medicine

"Let food be thy medicine and medicine be thy food" – Hippocrates (Father of modern medicine) [1].

"Food as medicine" is the philosophy long originated by Hippocrates which has been highly neglected [1]. For thousands of years, people all over the world focused on the benefits of edible food as a source of nutrition and medicine [2]. This development of edible food as medicine gave rise to traditional medicines [3]. Traditional medicine has developed throughout human evolution and now incorporates both edible and non-edible natural sources [4]. The benefit of naturally available edible sources is that they have very less side effects and are readily available in the household [5]. The traditional medicine may contain seeds, roots, shoots, leaves, barks, fruits, flowers, gums and resins of different plant species [6]. Therefore, the medicinally important plant species are cultivated and conserved traditionally [7].

1.2.Plant secondary metabolites

Different plant parts contain various phytochemicals (known as plant secondary metabolites) which are formed in response to biotic and abiotic stresses [8]. These plant secondary metabolites are grouped into terpenoids, phenolic compounds, alkaloids, glucosinolates and thiols [9]. As these organic compounds are produced mainly as a response to biotic stress, therefore they have bioactive properties such as antimicrobial, anticancer and anti-inflammatory activities [10].

The antibiological nature of these secondary metabolites also makes them toxic to humans at a high concentration, due to which standardization of the amount of intake of this traditional medicine is very crucial. In the Indian traditional medicinal system, ayurveda plays a vital role in documenting the methodology of preparing traditional medicines against various illnesses with minimal side effects [11]. Although ayurvedic medicinal preparation was found to have a significant role in enhancing the life quality of the patients, the use of toxic metals such as lead and arsenic in some of these preparations is a great concern in the scientific community [12, 13].

1.3.Traditional medicine and a brief introduction of garlic mustard oil macerate

Besides that, ayurveda has been able to incorporate thousands of different medicinal preparation procedures, but there are still various ethnomedicines available in different communities throughout India which are not been incorporated yet [14-16]. These ethnomedicine are used to treat various illnesses and their preparation methods are generally

transferred orally thus proper documentation is not available [17]. In Northeast India, garlic (*Allium sativum*) mustard (*Brassica nigra*) seed oil macerate (GMM) is an ethnomedicine that is used to treat the symptoms of the common cold such as nasal congestion and fever [18]. For the preparation of GMM, garlic cloves are crushed and then heated in mustard oil for a few minutes. The garlic to mustard oil ratio, heating temperature and time duration of heating vary among different communities due to which a standardized scientifically validated methodology is not yet documented. Although, garlic macerate in various other cooking oils along with the thiosulfinate compounds which are present in the formed are earlier reported.

1.4.Garlic (*Allium sativum*)

Garlic (*A. sativum* L.) is a bulbous, aromatic and herbaceous annual spice under the family of Alliaceae, which might grow to a height of 1.2 m [19]. Garlic presently is been cultivated throughout the world, but its origin is predicted to be in central Asia which then later moved to China, the Mediterranean region followed by Europe and Africa. It is one of the most valuable crop species due to its seasoning and flavour-enhancing qualities in culinary dishes. India is the second largest producer of garlic with a yearly production of 12,52000.00 metric tons after China with an annual production of 2,00,58,388.00 metric tons. In India, garlic is harvested during March and April. Due to the rapid fluctuation in the weather conditions of India, it is very difficult to maintain the quality of the garlic bulb and thus there is a serious challenge in the post-harvest techniques which are available presently. Mainly hard neck and soft neck garlic are more commonly used among various varieties and sub-species [20]. Many bioactive properties of garlic are already established such as anticancer, anti-inflammatory, antimicrobial, etc. [21, 22]. Garlic is known for its aromatic and volatile compounds which have been used in our food and medicines for more than 5000 years [23]. The use of garlic against common colds and inflammation is very common [24-26]. The major active principle of garlic allicin is known to have anti-inflammatory activity and trans-membrane potency along with a low boiling point [27, 28]. The bioactivity properties of garlic is mainly for its volatile organosulfur compounds such as allicin, diallyl sulfide (DAS), ajoene and vinyl dithiin [29].

1.5.Black mustard (*Brassica nigra*) and its chemical composition

B. nigra is a member of the Brassicaceae family. It is also known as black mustard and is called true mustard [30]. The word “mustard” originated from a European practice of mixing the mustard paste with the sweet ‘must’ of old wine which gives a hot paste (hot must), thus mustard [31]. It is an herbaceous and annual plant that can grow to a height of 1-2 m [32]. The leaf arrangement is petiolate, alternate and dark green hairy [32]. The lower leaves of the plant

are large. They have a small bright yellow colour cruciform flower with four petals, stamens tetradynamous and carpellate pistil [32]. The fruit is siliqua, smooth and quadrangular along with a short slender beak. Seeds are small and their colour may vary from red-brown to black [32, 33]. The seeds are comparatively much more pungent than the white or yellow mustard seeds [34]. It was found that Brassica species cultivation originated in the Middle East and Southeast Asia, where it was used for medical and cooking purposes [32]. Due to the high demand for *B. nigra* seeds for oil and medicinal purposes, a significant enhancement in its cultivation is observed globally [32, 35, 36]. The plant part of *B. nigra* is edible including its stem, inflorescence, leaves, seeds and roots. Under normal environmental conditions, the plant can give the maximum oil yield, but again under abiotic stresses like drought, waterlogging and high salinity the oil yield from the seeds of *B. nigra* may get reduced significantly [37]. Canada and the United States are the highest producer of *B. nigra* which is followed by India, the Mediterranean region, central Europe and the middle east [38]. Traditionally, mustard oil is extracted from the dried *B. nigra* seeds by cold press method [39, 40]. The oil is frequently used for cooking in India and other North-East Asian countries.

According to the reports USDA [41] (National Nutrient Database), 100 grams of mustard oil consists of the following constituents listed in Table no. 2. It was reported that *B. nigra* consists of chemical constituents which are very similar to *Brassica juncea*. The *B. nigra* seeds consist of Glucosinolates, which give the pungent smell of mustard paste. Sinigrin is the most abundant glucosinolate found in *B. nigra* seeds. The *B. nigra* seeds consist of the enzyme myrosinase, which is released after crushing the seeds and is responsible for the hydrolysis of sinigrin to allyl isothiocyanate (AITC) [42]. Therefore upon cold-press technique, the mustard oil consists of a high amount of AITC [43]. Mustard oil consists of omega-3-fatty acid which is considered effective to prevent colon cancer development in rats [44]. The various fatty acids identified in mustard oil were mainly oleic acid, linoleic acid, erucic acid and linoleic acid [45].

1.6.Biochemical and phytochemistry of garlic macerate in vegetable oils

The biochemical properties such as moisture content, ash and mineral content, carbohydrate, fibre, fat and protein of garlic cloves and mustard seeds were earlier reported [46, 47]. The biochemical properties of garlic cloves and mustard seeds determine the type of phytocompounds that may be present during the GMM preparation. As the oil of GMM is traditionally used during the treatment of the common cold, therefore more emphasis must be given to the physicochemical alteration of the oil post-preparation. Physicochemical analysis of oil consists of chemical properties (FFA, PV, AV, IV, SV) and physical properties (specific

gravity, density, viscosity, RI) which are crucial in determining the quality of edible oil [48]. During the preparation of GMM, heating of the oil at high temperature for a long duration is involved which may lead to a change in the quality of the oil and thus may not be suitable for human consumption or use.

For the phytochemical identification of the garlic macerated vegetable oil techniques such as FTIR, TLC, HPLC, LCMS and GCMS were reported to be used [49-51]. The nature and amount of the phytochemicals that are present in plant-derived medicines determine the effectiveness and dose during the treatment of the disease [52]. Therefore, the types of phytochemicals that are present in GMM are very crucial for its scientific establishment. Intact garlic has alliin which enzymatically gets converted to allicin due to the release of alliinase when the bulb is crushed [53, 54]. Allicin is highly unstable in both polar and non-polar solvents and as vegetable oils are non-polar therefore allicin gets converted to much higher stable organosulfur compounds (OSCs) which are ajoene, thioacroleins, 3-vinyl-4H-1,2-dithiin and 2-vinyl-4H-1,3-dithiin [53-56]. These garlic-derived compounds in non-polar solvents are known for their potent bioactive property against inflammation, various cancer cell lines, fungi and bacterial growth inhibition [18, 57-59].

1.7. Optimization of the traditional medicinal preparation

As traditional medicine preparations such as GMM are not documented in any literature, therefore the optimization of the preparation process is highly recommended. Optimization helps in standardizing the preparation process and in understanding the role of temperature, the ratio of mustard oil and garlic, the time for heating and the formation of various OSCs in mustard oil. Again, as the OSCs present in the GMM have antibacterial, antifungal and antioxidant properties, therefore the optimization of GMM can also be carried out concerning its bioactive properties. RSM is one of the best methods for the optimization process due to its statistical analysis and predictions [60]. RSM uses statistical and mathematical methodologies in the development, improvement and optimization of diverse processes along with actionable insights rapidly, without the hassle of multiple trials [60-62].

1.8. Traditional use of garlic mustard oil macerate and its bioactivity

Traditionally when the GMM is prepared in northeast India, the oil is put in the nostril, dorsum and alae of the nose to get relief from nasal congestion. Also, the oil is used for the massage of the palm, feet and back of the body which is also known to help in reducing fever-like symptoms. For reducing the nasal, NSAIDs are used due to their anti-inflammatory properties [63, 64]. Now, as GMM helps in reducing nasal congestion therefore hypothetically

the oil may also possess anti-inflammatory activity but very limited research has been conducted in this area. Again, the severe inflammation in the nose during the common cold often leads to sinusitis [65]. Sinusitis is an infection in the sinus region of the nose that may be caused mostly by viruses and secondary infections are caused by bacteria and fungi which give rise to diseases like acute purulent sinusitis and chronic sinusitis [66]. In patients with immunodeficiency, sinusitis may get adverse and thus it is very essential to control the infection and inflammation caused by the common cold [67]. After the use of GMM oil such infections are not observed in the patients therefore the oil may also possess both antifungal and antibacterial properties. As the GMM oil is lipophilic therefore during massage maybe the compounds present in the oil may pass down the stratum corneum and reach the bloodstream which may also help for the reduction of inflammation. Thus, for the scientific establishment of the traditional practice of using GMM during the common cold, research in compound identification in GMM, process optimization and its role against bacteria, fungus and inflammation is the utmost priority.

Secondary bacterial infections during the common cold are very common and are caused by bacterial species like *Streptococcus pneumoniae, S. aureus and P. aeruginosa* [68]. Therefore, investigating the bioactivity of GMM against both gram-positive and gram-negative bacteria is very essential. The antibacterial property of a substance is regarded as its ability to hinder and irradicate bacterial growth [69]. These properties make antibiotics very important in the field of healthcare and the food preservation industry [70, 71]. There are two aspects of antibacterial agents, namely bacteriostatic (kill bacteria) and bactericidal (inhibit bacterial growth) properties [72]. Although, the discovery of antibiotics has revolutionized the approach to the treatment of life-threatening bacterial infections [73]. The misuse of antibiotics also gave rise to resistance to the bacteria, which is presently one of the major global challenges [74]. Thus, the finding of new antibacterial compounds from natural compounds, nanoparticles, or novel synthetic drugs for the combat against the bacteria is the need of the hour.

During the common cold, *P. aeruginosa* and *S. aureus* are found to cause secondary infection and form biofilm which gives them protection against antibiotics [68]. Therefore, the anti-biofilm and quorum sensing inhibition property of GMM is also an interesting field to be explored. Biofilm is the complex amalgamation of microbes (mainly bacteria) and self-producing extracellular polymeric substance (EPS) which consists of proteins, polysaccharides and nucleic acids [75]. The adherent nature of the biofilm in medical devices (such as catheters

and implants), body tissue and food sources makes it very difficult to get removed [76]. The biofilm acts as a protective shield for the bacterial cells (sessile cells) against antibiotics which cannot penetrate through the biofilm then compared to the free-floating planktonic cells outside the biofilm [77]. As the antibiotics cannot penetrate the biofilm therefore it makes the fight against bacterial infection much more challenging [78]. The present approaches against biofilm formation include mechanical, chemical and biological methods [79]. Under the chemical strategy, compounds having the ability to inhibit quorum sensing, having enzymatic activity such as DNase and protease, or as surfactants that may disrupt the biofilm matrix are selected and studied [80, 81].

Like secondary bacterial infections, fungal species like *Candida albicans* are also reported to cause severe infections during the common cold in immunocompromised patients [82]. Fungus is very difficult to kill as it is a eukaryotic organism and the commercially available antifungal drugs are reported to be highly cytotoxic to humans [83]. This fundamental problem against fungal treatment is a matter of great concern in the field of medical science. Therefore, edible plant-based drugs having antifungal properties are highly recommended for the treatment of fungal infections in the upper respiratory tract [84]. Therefore, as the GMM is a food-based medicinal preparation and thus its potential against fungal growth inhibition can be explored.

During the common cold, the nasal mucosal lining is infected by viruses (rhinovirus, influenza virus, coronaviruses) which trigger the body's immune response against the virus [85]. After the activation of the immune system, pro-inflammatory cytokines (such as IL-1, IL-6, IL-8 and TNF-α) are released which attracts macrophages and neutrophils to the infected area [86, 87]. The inflammatory mediators also lead to vasodilation which increases permeability of the blood capillaries in the nasal mucosa [88]. The increased permeability leads to fluid leakage, thus resulting in nasal congestion and a runny nose [88]. This rapid production of immune cells and fluid causes swelling of the nasal mucosa and edema which causes nasal blockage and difficulty in breathing [89]. Inflammation is a complicated biological mechanism of the immune system that gets activated under the stimulus of pathogens, infection and irritants to protect the body from further infection and help in healing [90, 91]. The inflammatory response in the body can be identified by signs such as redness, pain, swelling and heat [92]. But prolonged inflammation may lead to chronic inflammation which may again lead to the development of metabolic disorders like diabetes, cancer and cardiovascular diseases [93].

Traditionally, GMM oil is used for massage in patients suffering from the common cold, therefore the transdermal study is necessary for understanding the penetration of the active molecule through the stratum corneum. The transdermal drug delivery system (TDDS) is an approach by which active compounds are absorbed through the protective barrier of the skin (stratum corneum) and reach the bloodstream for systemic drug delivery [94]. Due to the property of non-invasive nature and avoiding the metabolism of the drug by the liver, TDDS is considered more advantageous than conventional drug delivery methods such as oral and intravenous approaches [95]. For drug delivery by TDDS, strategies such as the use of chemical enhancers and microneedles in the patches which help in the increased penetration of the drug molecules are used [96, 97].

1.9. Gap in Study

The gap in the study includes:

1. The compounds present in the GMM oil preparation are not known.
2. The optimum condition for the preparation of GMM is not yet established.
3. Earlier research on the bioactivity of GMM against bacteria, fungi, viruses and inflammation is not well studied.

1.10. Objectives of the present research

In the light of above information and the gap in research, we formulate the following objectives for our research:

Objective 1: Physicochemical analysis of garlic (*A. sativum* L.) cloves, mustard (*B nigra* L.) seeds and garlic mustard oil macerate along with compound identification.

Objective 2: Optimization of garlic (*A. sativum* L.) mustard (*B. nigra* L.) oil macerate.

Objective 3: Evaluation of the potential of the optimized garlic (*A. sativum* L.) mustard (*B. nigra* L.) oil macerate in antibacterial, antibiofilm, antifungal, anti-inflammatory and transdermal activity.

1.11. References

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