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Formulation, Characterization, and Microbial Studies of Herbal Remedy for Asthma Patients

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Asthma is a lung inflammatory disease present all over the world. Environmental conditions and genetic factors play a critical role in the progress and management of this disease. In Pakistan, the air pollution index increases rapidly especially smog intensify the severity of asthma. Patients use corticosteroids to overcome asthma allergens that cause negative effects on the body. Keeping in view the present investigation is based on herbal remedies i.e., herbal infusion tea, formulated from a natural source. These are made from herbs such as black seed, lemongrass, viola, ginger, and cardamom. The main bioactive compound in the herbal infusion tea was thymoquinone. There were three different formulations (F1, F2, and F3) made. Chemical analysis, water activity, pH, detection of thymoquinone, microbiological studies, and sensory evaluation were performed. The bioactive compound was detected by a fourier transform infrared analyzer at 1636.05 and 2340.87 and microbiological studies show that after the infusion of tea in boiled water, all forms of microbes were removed and safe for drinking. F3 formulation was the best formulation selected based on the sensory evaluation that contains moisture 8.50%±0.32, ash 8.30%±0.82, cured fiber 26.35%±1.30, crude fat 8.20%±0.35, total solid 91.50%±0.82, water activity0.60±0.03 and pH 5.33±0.04. This study was conducted on 10 patients, 7 out of 10 given positive responses and feel batter in their health. Herbal infusion teas are the most effective product for asthma patients and store a long period of time. Herbal formulation has the ability to cope with asthma allergens and does not cause side effects on the body.

Keywords: Asthmatic Formulation, Thymoquinone, Fourier Transform Infrared, Sensory Evaluation, Shelf Life.

Introduction

Asthma is a chronic respiratory tract disease characterized by prolonged cough, shortness of breath, and wheezing (Al-Tufaily, Hussein, & Hussein, 2022). Asthma affects 330 million people worldwide (Szefler et al., 2014) and additionally 100 million are expected to have asthma by the year 2025 (Sabar et al., 2018). Air pollution has an adverse effect on human health. Extremely small particles present in polluted air can travel into the human respiratory tract and penetrate deeply into the epithelial cell of the lungs and cause asthmatic symptoms. The degree of severity of asthma is determined by the amount of airflow limitation and speed with which the situation is reversed (Ho et al., 2024). About 2 million deaths have been reported annually in the world so the WHO declared air pollution is a "Silent Killer" (WHO, 2018).

Environmental factors increase the severity of asthma (Dakhama et al., 2009). Allergens are environmental antigen has the ability to induce the production of specific IgE antibodies. IgE is a specific type of Immunoglobulin (Froidure et al., 2016). Strongly associated with type -1 hypersensitivity reaction with its low level of plasma and short half-life. In addition to lateral phase response and activating immediate-hypersensitivity reaction, there is accumulating sign that performed IgE can augment the cellular and humoral immune response to allergens (Cockcroft et al., 2005).

The main objective of asthma management is to resolve the symptoms caused by an asthmatic attack with minimum use of anti-asthmatic drugs to ensure less or no side effects of drugs. The management not only includes drug therapy but some other feature like adjustment of lifestyle, diet, and exercise also very necessary (Kling & White, 2021). Bronchodilators and corticosteroids are frequently used for the treatment of asthma. Systematic corticosteroids play a vital role in the management of bronchospasm, but the medicines have a serious effect on the health of patients. Hyperglycemia, cardiovascular disease, osteoporosis, Cushing's syndrome, neurological disorder, and immunological suppression are frequently reported during the prolonged use of the remedies (Dahl, 2006; Nguyen, Hoang, & Nguyen, 2022). Fungal infection and harshness are the most common side effect reported in a patient who inhaled bronchodilators and corticosteroids (Barnes, 2010).

The advantages of herbal formulations over other chemical drugs and remedies are low risk of side effects, formula medicine cause side effects when they were used regularly. These are most effective for the treatment of diseases. The cost of this formulation is very low and easily available in the local market. These are accessible in large amount. Simply some are cultivated in-house and used for treatment (Punpromthada et al., 2022; Rane et al., 2021).

The research focuses on the extraction of the bioactive compound that interacts with IgE to control asthma symptom. Thymoquinone is a bioactive compound that could act as an antihistamine (Ahmad et al., 2021). The compound is present in black seed also known as a *Nigella sativa* seed belong to Ranunculaceae family, which is a traditional herb and spice that are used to cure especially

bronchial asthma. Research showed that boiled extract of black seeds productively improved asthma-related messes in asthmatic patients. In a comparative report, fifteen patients suffering from asthma were given boiled extract which reveals to improvement in asthma symptom response (Boskabady, Mohsenpoor, & Takaloo, 2010; Jiang, 2024). There are various chemical constitutes such as thymoquinone (30%-48%), thymohydroquinone, p-cymene dihymoquinone (7%-15%), carvacrol (6%-12%), sesquiterpene longifolene (1%-8%) 4-terpinol (2%-7%), tenthol (1%-4%) nigellimine-N-oxide, nigellicine and nigellidine. These compounds show many benefits on human health (Sultana et al., 2015).

Lemongrass is present in the phytogenic substance and its scientific name is *Cymbopogon citratus* (Golo, McApreko, & Quarshie, 2023; Ranade & Thiagarajan, 2015). It is widely distributed worldwide and belongs to the Poaceae family. It has been widely consumed due to medical, nutritional, cosmetic and gives a pleasant aroma and taste to food. There are many pharmacological properties present in lemongrass. The medicinal properties of lemongrass and its oil give many health benefits (Alagawany et al., 2021). Lemongrass leaf is traditionally used in KAHVA or infusion tea (Toungos, 2019). It has many flavonoids and they act as an anti-inflammatory compound (Avoseh et al., 2015; Jethwani & Ramchandani, 2022).

Ginger belongs to the Zingiberaceae family. Its scientific name is *Zingiber officinale*. Worldwide use in beverage and dietary condiments in food (Ujang, Nordin, & Subramaniam, 2015). Ginger is a nutritional plant and is effectively used in medicine. Ginger has some general compounds such as Fe, Mg, Ca, Vitamin C, phenolic compounds (Gingerdiol, gingerol, gingerdione, and shogaols) flavonoids, sesquiterpenes, parasols which were used in herbal medicine (Shahrajabian, Sun, & Cheng, 2019). Scientific studies showed that ginger helps to reduce inflammation and pain (Singletary, 2010).

Cardamom belongs to the Zingiberaceae family and its scientific name is *Elettaria cardamomum L* (Jesylne et al., 2016). It has been used in many medicines, due to various medicinal properties. Cardamom is effective for the treatment of Asthma and relaxes the airway (Habash, Sultan, & Ghareeb, 2022; Qiblawi et al., 2020). Phytochemical investigations have depicted significant synthetic constituents of cardamom including carbs, proteins, minerals, lipids, fundamental oils, flavonoids, terpenoids, and carotenoids. Some major advantages in health including cancer prevention agent, antidiabetic, antibacterial, anticancer, gastro-defensive, and insecticidal exercises (Ashokkumar et al., 2020).

Viola belongs to the Violaceae family. Its scientific name is *Viola botonicifolia* and locally known as Banafshe, it is widely distributed throughout the world including Pakistan. The plant leaves and fruits have traditionally been used for the treatment of bronchial asthma, bronchitis, and cough (Janbaz et al., 2015; Misra et al., 2022). The anti-inflammatory properties were checked by using an aqueous extract of viola adorate and compare with hydrocortisone in the rat by induction of lung injury by formalin through nebulization. These are very effective parts in avoiding lung damage and they could be used as an alternative corticosteroid to the management of the inflammatory condition of the lungs (Bashir et al., 2021; Llamazares, 2023).

There are many medicines in market but not any herbal related to cope the asthma. Our product is proved beneficial in these days when smog severity is high. It's easily accessible in local market on cheap rate. The main purpose of this research is to develope a herbal formulation for asthmatic patients as per our best knowledge no other studies have been investigated before.

Materials and Methods

Collection of Raw Material

The selected herbs were the following: black seed, lemongrass, viola, ginger, and cardamom. These herbs were collected from the local market. Lemon Grass has been taken from the National tea and high-value Crop research institute Shinkiari Mansehra. Other herbs like the black seed, viola, ginger, and cardamom were purchased from the local market in Lahore. The collected herbs were preserved in air-tight polythene bags. Tea bags were bought from the local market.

Raw Materials Analysis

Before the formulation, chemical analysis was performed such as moisture, crude fat, crude fiber, total solid, ash, etc. The representative sample taken from each ingredient such as leaves and seeds has been ground into powder form before analysis. The analysis was performed by using standard techniques provided by AOC (Nielsen, 2010).

Moisture and total solid content were determined by drying the sample at 100°C in a hot air oven up to constant weight (Younas et al., 2021). Ash content was determined by igniting the sample at 525°C in the furnace up to constant weight (Mafu et al., 2022). Crud fat was determined by diethyl either extract in Soxhlet apparatus at 40-60°C then evaporate the solvent from the sample (Azevedo et al., 2022). Crud fiber was determined in the defatted sample by 0.125N sulfuric acid and 0.313N sodium hydroxide digestion. After digestion, the ash content was determined for crude fiber (Salarzadeh et al., 2012).

Formulation of Infusion Tea

Herbal infusion tea was made into three formulations. These were given the names F1, F2, and F3. In these formulations, some ingredients were kept constant, and some were variable for test perception. Constant ingredients are black seed, ginger, and cardamom and variable ingredients are lemongrass and viola.

Formulation Analysis

Chemical analysis was performed for each analysis. These are the following analysis performed moisture, crude fat, crude fiber, total solid, ash, and pH. These were analyzed according to the stander method of AOAC (Nielsen, 2010).

Water activity

A representative sample was analyzed in the water activity meter. The weight samples were kept in the water activity

analyzer at 25°C and note the reading (Sarkar et al., 2022).

pH Analysis

pH meter (Seven compact S-220) was used to determine the pH of a formulation (Ijaz et al., 2022).

Microbiological Analysis

2g of sample were taken in the conical flask containing 200ml 0.1% ringer solution and kept at room temperature for 1 hour. 10-fold serial dilution was prepared. Similarly, one of the samples were taken into boiling water and allowed to boil for 5 minutes on the hot plate. The beaker was removed from the hot plate and kept for 30 minutes at room temperature under aseptic conditions. 10- Folds serial dilution was prepared (Etim, Simonyan, & Eke, 2022). For total plate count 1ml sample was taken from 10-serial diluted sample and inoculated into the nutrient media plate by spreading method. The sample was put into the incubator at 37°C for 48 hours. The colony was counted by colony meter (Hoque, 2013). For Yeast and mold 1ml sample was taken from 10-serial diluted sample and inoculated into potatoes dextrose agar media plate by spreading method and incubator at 37°C for 78 hours (Greppi et al., 2013). For coliform 1ml sample was poured into the plate. Then violent red ager media was poured into the sample. The media containing the sample was kept for cooling and solidification. Then the plate was incubated at 37°C for 24 hours. The colony was counted in colony meters (Le et al., 2022).

Sensory Evaluation

To determine the best formulation of infusion tea. Sensorial evaluations of F1, F2, and F3 were carried out by Sami-trained panelists. All samples with different compositions of herbs were presented to the Sami-trained panelist and noted the feedback. A simple hedonic scale was used, which consist of five attributes such as color, aroma taste, flavor, and overall acceptance (Wang et al., 2022).

FTIR Analysis

The tea bag was put into 200ml distilled water at 100°C and stirred for 5-8 minutes. A drop of the extracted sample was added to the surface of the KBr pellet on FTIR analyzer. FTIR spectrum of the Infusion tea in KBr pellets (V max in cm-1) on an FTIR spectrum of Bruker FTIR spectrometer in the wave range of 600-4000cm-1 with the resolution of 2cm-1 (Mohamed Shameer & Mohamed Nishath, 2019).

In-Vivo Studies

Select 10 people of the same age and same group. They would be given him for 15 days regularly and then the feedback has been taken from the patient and asked the following Question regarding her health (Wu & Wei, 2009).

Shelf-life

The product was kept in a humid chamber, the humidity was set at 65-75% and the temperature was kept at 40-45°C. One of the products was kept at room temperature. After 3 months, the sensory evaluation was performed Compare both formulations that are kept at room temperature and humid chamber by the sensory panelist (Steele, 2004).

Results and Discussion

Table 1 shown the raw material analysis in which, Lemongrass was the main ingredient used in herbal infusion tea. The moisture content was 7.67%. lemongrass has been taken in dried form. Moisture content was changed due to various factors such as the dried and storage condition of lemongrass. Moisture contents were effect by other values such as ash, total solid, curd fat, crude fiber, and water activity. The percentage value was increased and decreased due to a change in moisture content (Unuigbe et al., 2019).Black seeds were analyzed by stander method. All these results obtained by the analysis of black seed were accurate and within the range of stander results. The results were obtained such as moisture content 5.11%, ash 15.33%, total solid 89.33%, curd fat 32.82% crude fiber 16.43%, and water activity 0.577 (Odebunmi, Oluwaniyi, & Bashiru, 2010).

Viola has great pharmaceutical potential. The stander method was used and results were obtained such as moisture content 10.67%, ash 11.0%, total solid 89.33%, curd fat 4.23% and crude fiber 30.10%, and water activity 0.60. The moisture content was changed due to various factors such as storage and dried condition of viola leaves (Barkah et al., 2021).

In the present study, cardamom was analyzed. The following results were obtained such as moisture content 13.33%, ash 8.67%, total solid 86.7%, curd fat 3.22%, crud fiber23.00%, and water activity 0.56. All the values obtained are compositionally different. The compositional variation in cardamom samples concerning physicochemical composition depends on varietal differences, agronomic practice, and climate conditions. Moreover, the stage of production and maturity is also an important factor that affects its physicochemical composition parameter (Muhammad et al., 2012). Ginger was dried in a hot air oven and made the powder. The proximate value was obtained such as moisture content 9.67%, ash 5.67%, total solid 90.33%, curd fat 4.3%, crude fiber 5.63%, and water activity 0.56. Crude Fat and water composition were changed due to various factors such drying method of ginger and the storage condition of ginger (Kumari & Gupta, 2016).

Table 1: Chemical Analysis of Raw Materials.

No	Herbs	Moisture (%)	Total Solid (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)
1	Lemon grass	7.67±0.58	92.33±0.58	8.67±0.58	4.17±0.29	39.80±3.00
2	Black seed	5.11±0.50	94.89±0.26	15.33±0.58	32.82±0.68	16.43±0.52
3	Viola	10.67 ±058	89.33±0.58	11.0 ±0.01	4.23 ±0.23	30.10±1.31
4	Ginger	9.67 ±1.15	90.33±1.15	5.67 ±0.58	4.30 ±0.33	5.63±0.15
5	Cardamom	13.33±1.15	86.7 ±1.15	8.67 ±0.58	3.22 ±0.12	23.00±1.10

Chemical analysis was shown in Table 2. The minimum activity of food-born pathogen growth is from 0.83 to 0.86

(Troller, 2017). The product is placed in less than this range which is in a safe zone. If the water activity is more than 1 the product is prone to survive the microbes. The microbial activity depends upon the water activity of the product. All these formulations have less than 0.83 water activity. It means that all of them were in the safe zone.

pH was shown in table-2. pH was the main important part of any formulation which played an important role in the stability and taste of any food material. Formulation no 3 has a pH of 5.3. it is a miner difference in pH among all the formulations.

All the ingredients used in the formulation in which had the ability to antimicrobial and some antioxidant activity. Each formulation was shown different results. The formulation that was made before boiling water contains few microbial contaminations in total plate count and coliform that are given in table-3 these are below o stander of microbial load. The following table shows the result of different formulation microbial activities. All the microbes were killed at high temperatures. Table 3 showed the result of microbiological quality. In all formulations, there was no sign of microbes, when a person consumed. It clearly showed that there was no microbial contamination of the product. One of the other reasons for a low rate of microbiological was that the moisture content of every formulation is less than 10% approximately between 7% - 8.5%. And the water activity of each formulation was less than 0.80 (Untermann, 1998).

Formulation no 3 was selected best one, according to Figure 1, the overall acceptance of the product was given by Formulation no3, and all others were not meet the corner of the defined scale. People were given positive feedback by used of Herbal infusion tea because they were well known about the side effect of herbal formulation as compared to corticosteroid.

The spectrum was obtained from the FTIR analyzer shown in Figure 2. Obtain peaks were compared with stander peaks. In this spectrum, C =C stretching strong at 1636.05 indicate alkene, N=N=N stretching strong at 2130.95 indicate azide, O=C=O stretching strong at 2340.87 presence of carbon dioxide , and at 2340.87 N-H shows medium stretching representing secondary amine (Mohammed et al., 2019).

Table 2: Comparison of Chemical Analysis of Different Formulations.

No	Formulation	Moisture (%)	Ash (%)	Total Solid (%)	Crude Fat (%)	Crude Fiber (%)	water activity	pH
1	Formulation 1	8.55±0.05	8.35±0.05	91.45±0.05	6.15±0.05	28.80±0.10	0.6149±0.03	5.24±0.03
2	Formulation 2	7.90±0.05	8.00±0.68	92.10±0.05	6.90±0.40	24.10±0.12	0.6170±0.04	5.23±0.04
3	Formulation 3	8.50±0.32	8.30±0.82	91.50±0.82	8.20±0.35	26.35±1.30	0.6082±0.03	5.33±0.03

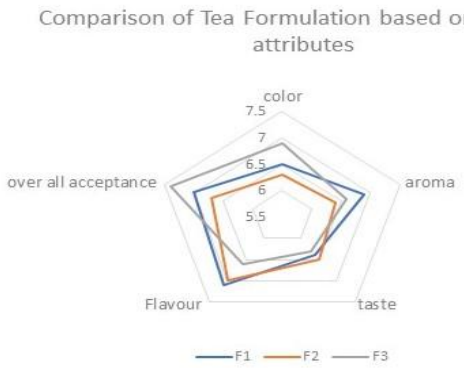

Figure 1: Comparison of F1, F2 and F3.

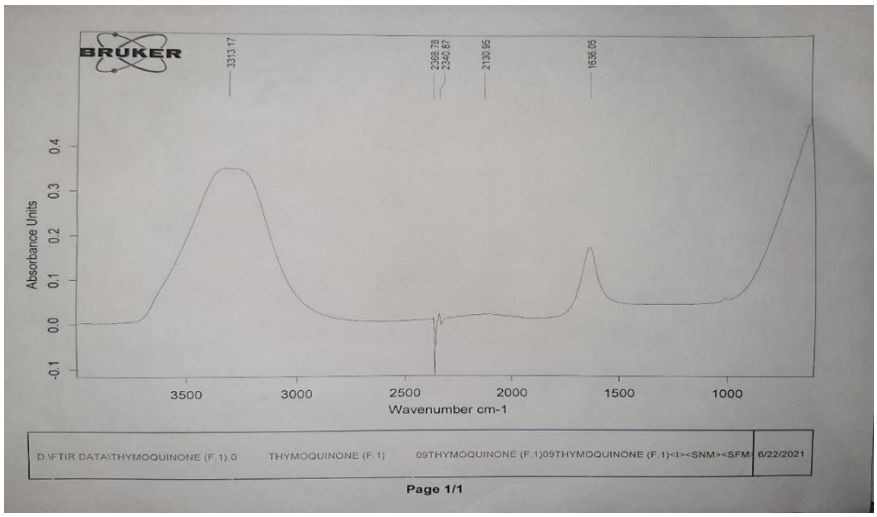

Figure 2: Spectrum Obtain from FTIR.

Table 3: Microbiological Content Before Boiling and After Boling Water.

No.	Formulations	Total plate(cfu)		Coliform(cfu)		Yeast (cfu)		Mold(cfu)	
		Before	After	Before	After	Before	After	Before	After
1	Formulation 1	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2	Formulation 2	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3	Formulation 3	+ve (2cfu)	-ve	+ve (2cfu)	-ve	-ve	-ve	-ve	-ve

Herbal Infusion tea has been taken for 15 days regularly. After 15 days, they gave feedback about the infusion tea. Out of 10, 7 were given positive responses and, 3 were not given any response about the improvement of Asthmatic health. Figure 3 showed the patient remarks.

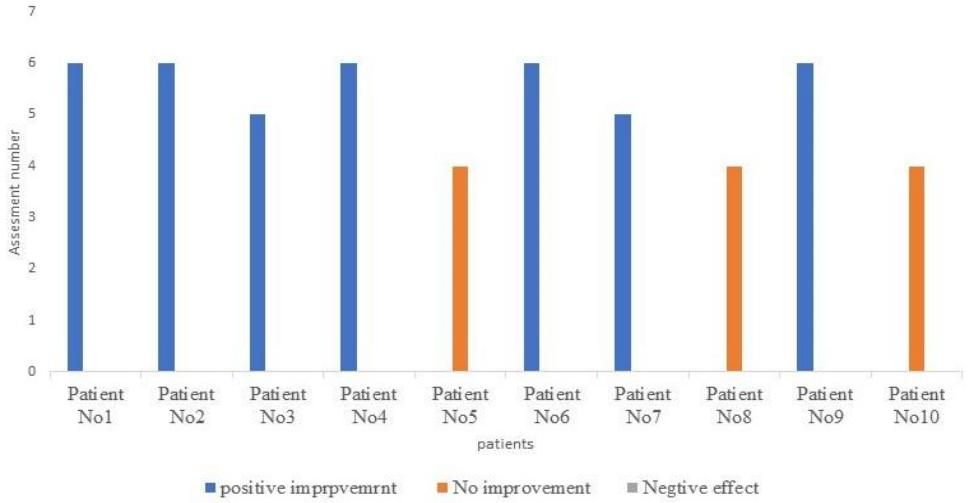

Figure 3: Patients Was Given Feedback About Herbal Infusion Tea to Improve Their Asthmatic Health.

After the 3-month sensory evaluation has been done. This shows that there was not any difference found in taste, aroma, and flavor. During the study period, the product shelf-life assign 3-month but based on its composition it should be kept at 1 year or more because it has a low moisture content, water activity, and pH. Under specific temperatures and good packaging prolong the shelf life of infusion tea. The comparison of these were shown in Figure 4.

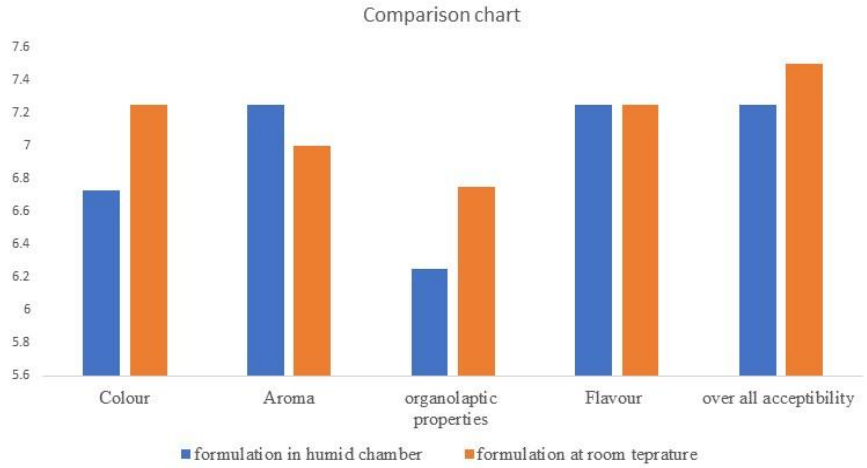

Figure 4: Comparison Chart in The Humid Chamber and at Room Temperature.

Conclusion

Air pollution and smog increase day by day in Pakistan which cause an adverse effect on the asthmatic patient. The use of corticosteroids causes a negative effect on the body. The herbal infusion tea is the best remedy for an asthmatic patient because it contains many different bioactive compounds. Thymoquinone is a bioactive compound found in black seed and is effective for asthma patients. Three formulations were made F1, F2, and F3. Comparing all the formulations, F3 was selected as the best one. It contains the potential to reduce asthma with good sensory attributes.

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