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# IDENTIFICATION AND ISOLATION OF BIOACTIVE COMPONENTS USING GC-MS, UV VISIBLE, FTIR, HPLC, HPTLC AND ANTIOXIDANT ACTIVITY PRESENT IN ETHANOLIC EXTRACT OF INDIAN SPICES MIXTURE

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**Keywords:**

Flavanoids, Quercetin, Rutin, Antioxidant activity, GC-MS, UV, FTIR, HPLC, HPTLC

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**ABSTRACT: Introduction:** The spices, despite their taste and flavor, have certain therapeutical uses from ancient days. For this research work, we have chosen some selected spices indefinitely amount. **Materials and Methods:** Phytochemical analysis, mineral estimation Characterisations such as GC-MS, UV Visible & FTIR spectroscopy, HPLC, HPTLC, and Antioxidant activity were carried out. **Results & Discussion:** Results of the research have shown that the phytochemicals like Flavonoids, Phenols, and Proteins were found in abundance, Alkaloids, Saponins, Terpenoids, Anthocyanins, Carbohydrates, Xanthoproteins, and Cardiac glycosides are moderately present in the mixture extract and Tannins, Phlobatannins, Coumarins, Emodin, Lekoanthocyanin, Anthroquinone, steroid, Glycosides are present in trace amount. In this study, minerals such as Iron, Sulphur, Manganese, Sodium, Organic carbon, and Zinc were abundantly present in the spices mixture extract. Copper and Phosphorus were moderately present. Calcium, Potassium, Magnesium, Nitrogen, Boron, Selenium, and Molybdenum were present in trace amounts in the mixture extract. Spices mixture extract is a rich source of antioxidant activity. The DPPH assay and total antioxidant activity of spices were carried out, and the results were obtained, indicating the strong antioxidant activity. Through GC-MS, 45 compounds were obtained, and in these 12 compounds were proven biologically active using Dukes database. Functional groups present in the extract were identified using UV Visible and FTIR Spectroscopy was done to identify the σ-bonds, π-bonds, and lone pair of electrons of the identified components. HPLC was carried out to determine the predominant flavonoids like Quercetin, Rutin, Gallic acid, and Catechin. HPTLC was performed to estimate the Quantity of the flavonoids, especially Quercetin and Rutin. **Conclusion:** Due to Flavanoids, Minerals and Antioxidants, this study proves to have a strong curative effect against respiratory infections.

**INTRODUCTION:** Respiratory Tract Infections are infections caused by any microorganisms in the upper or lower part of the respiratory tract. These are the most common and one of the leading problems in developing countries [1]. Generally, infectious microorganisms affects the respiratory system includes nostrils, trachea, sinuses, throat, bronchus, lungs, and alveolus [2] and causes the common cold, laryngitis, tonsillitis, acute rhinitis, acute rhinosinusitis, and acute otitis media in the Upper region.

The lower mode includes acute bronchitis, bronchiolitis, pneumonia, and tracheitis [3]. Generally, microorganisms cause common respiratory infections in daily life at all ages and seasons. Pathogens enter the distal airway by inhalation, aspiration, or hematogenous seeding and multiply in or on the epithelium. Infections spread when a person breathes air containing

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droplets that were coughed or sneezed out by an infected person [4]. Respiratory infections are cough, sneezing, stuffy or runny nose, sore throat, headaches, muscle aches, breathlessness, tight chest or wheezing, high temperature, and feeling generally tired [5]. Viral pathogens are the most common cause of respiratory infection [5]. The entry of pathogens may cause redness, edema, hemorrhage, inflammation, increased mucus secretion, impaired mucociliary function, severe bronchiolitis, inflammation, and necrosis of the epithelium may block small airways leading to airway obstruction [6, 7].

Chemotherapeutic drugs are used to treat respiratory infections which have certain complications such as Allergic reactions - asthma, Hypersensitivity pneumonitis, Eosinophilic pneumonia, Alveolar hemorrhage, Bronchitis, Interstitial fibrosis, lupus erythematosus, Granulomatous lung disease, Pneumonitis or infiltration, Lung vasculitis, Lymph node swelling, Mediastinitis, Pulmonary edema, Pleural effusion and Pulmonary hypertension [8]. To avoid the side effects, herbal medicines have long been used to treat and prevent viral respiratory infections (VRI) [9]. In the olden days, respiratory infections were treated with medicinal plants without any side effects, termed phytotherapeutic agents with pharmacological properties. Herbs and spices have been used in many different ways.

Since ancient times, spices and culinary herbs have been added to food to enhance flavor and improve their organoleptic properties and are widely used as preservatives and medicines. People have used spices which are aromatic plant substances whose significant functions in seasoning rather than nutrition in food for many generations to cure various ailments [10]. Herbal medicines play an important role in preventing and treating respiratory infections and are useful for treating the common cold, viral pharyngitis, acute bronchitis, and influenza. Herbs for treating such infections will be considered first, including both traditional treatments and those that have been scientifically researched [11]. In Ayurveda, spices and herbs have distinctive functions like carminative properties, cold & cough relief, diuretic action, and cardiac stimulation [10]. With the support of literature, In our study, we have chosen some specific Indian spices, such as Cinnamon, Cardamom, Clove, Cumin, and Pepper which have strong actions against respiratory infections. Cinnamon bark is used for treating gastrointestinal upset, diarrohea, stimulating appetite, menstrual cramps, allergies, common cold infections, sore throats, cough, indigestion, abdominal cramps and flatulence and intestinal spasms, influenza, food poisoning, reduced risk of heart disease, lowers blood sugar level, powerful Antidiabetic effect [12]. It helps to fight against bacterial and fungal infections, tooth decay and heart diseases. Cinnamonis beneficial for stop acne, reduce inflammation and aging brain. It can also enhances the freshen breath and also acts as antioxidant plump [13].

Cardamom is used for digestion problems including heartburn, Irrita, Bowel Syndrome, Constipation, maintaining high blood pressure, blood circulations, acts as anti-cancer, antidiabetic agent. It fights against asthma, cough, Sore throat, Headache, and Bronchitis. Improves fresh breathening, maintains the oxygen level and mind relaxation [14]. Cardamom helps to treat urinary disorders, body detoxification, joint pain, depression, Hypertension [12]. Clove decreases the number of harmful bacteria more than the commercial mouth rinse. Cloves are meant for their potential effect on obesity, reduce arthritis, and inflammatory response in the body.

It is also helpful to protect the stomach from ulcers and promotes better liver function [15]. Clove contains an important nutrient, Manganese which is essential for maintaining brain function and building strong borne. They are high in antioxidants compounds that reduce oxidative stress, protect against cancer, improve liver health, regulate blood sugar and the digestive system, and improve respiratory tract health. Clove cleans the tongue and palate upper part of the throat of any bacteria, diabetes management, liver protection, boosts immunity, improves hair health, and maintains respiratory health [16].

Cumin helps improve the digestion, boost memory, boosts the metabolism, lowers cholesterol level, helps to decrease blood sugar, improves the ratio of HDL and decreases the LDL cholesterol, Supports the immune system, and has cancer-fighting properties; treats Menstrual problems, Diarrhea,

Colic, Bowel spasms, and Gastric problems, Used to increase urine flow to relieve bloating to start menstruation, and A diet high in antioxidant can help reduce the risk of heart disease and some other chronic disease [12]. Treatment for gastrointestinal distress, jaundice, and respiratory disorders Reducing oxidative stress Suppressing inflammatory marker expression Modulating signaling pathways controlling cell death, and Altering circulating hormone levels [17, 12]. Pepper is used to treating arthritis, asthma, stomach upset, bronchitis, menstrual pain, stuffy nose, sinus infection, dizziness, weight loss, cancer, fever, and malaria [18].

Pepper is used for respiratory disease and also preventive for rheumatism, stuffy joints and cheats cold with cough and heart arrhythmias, heart disease, abdominal, constipation, tooth decay, liver disorders, lung disease, and indigestion, Helps to control blood sugar and lower cholesterol levels, Helps in the absorption of nutrients. Enhances the digestive tract, Relieves joint pain. Mitigates migraines, Improves metabolism, fights fungal infection. Reduces risk of Cancer, Prevents bad breath, allergies, helps maintain blood pressure, skin health, hair growth, and brain health [19]. This research aims to identify, analyze, and isolate the bioactive compounds in the spices extract mixture, which helps to cure respiratory tract infections.

## MATERIALS AND METHODS:

**Collection of Spices Sample and Preparation of Ethanolic Mixture Extract:** The spices are naturally available in hilly areas of various places. The spices Clove, Cinnamon and pepper were collected from Kolli hills, Trichy district, Tamil Nadu and the spices Cardamom and Cumin were collected from kumily district in dried condition. The collected spices were shadow dried for another 3-5 days. The dried specimens were made a fine powder using a grinder mixture and stored in an air-tight container. The 10g powder material of the sample was mixed with 100ml of Ethanol at room temperature and kept in Hot perculator for about 30 minutes. After 30 minutes, the mixture was filtered and the solution alone was collected in a separate conical flask. The extract obtained from the above method was stored in a glass vial for further processes (20). The prepared extract was subjected to Qualitative estimation of phytochemicals. Qualitative and estimation of minerals, identification of bioactive compounds by GCMS method [20]. Identification of bonds and functional groups by UV-Visible and FTIR spectroscopy [20]. Identification of flavonoids by HPLC method [21]. Quantification of identified flavonoids by HPTLC method [22] and *in-vitro* Antioxidant activity [20].

**RESULTS AND DISCUSSION:** In this present study, the spices mixture extract was subjected to qualitative analysis of phytochemicals and represented in **Table 1A**. Flavonoids and Phenols are found to be abundant, while Alkaloids, Terpenoids, Saponins, Anthocyannins, Carbohydrates, Xanthoproteins, Cardiac glyciosides were present in moderate levels. Tannin, Phlobattanins, Coumarins, Emodins, Leucoanthocyanin, Anthroquinone, Steroids and Glycosides were present in trace amount. Selectively total flavonoids, total alkaloids, lignin, tannin, serpentine, and glycosides were estimated quantitatively and given in **Table 1B.**

Alkaloids possess plasmolytic, anti-cholinergic, analgesic, antimalarials, and anesthetic activity, reducing fever and headache. Alkaloids act as cardiac or respiratory stimulates [23]. Flavonoids are especially known for their antioxidant activities, which play a significant role in cardiovascular health and prevention. They are also known for their antihistamine, anti-microbial, memory enhancing, and even mood-boosting properties. Oxidative stress in the respiratory tract leads to the generation of arbitrators of lung infiltration and instigate the process of carcinogenesis [24]. Flavonoids have a certain protective effect on (COPD) chronic obstructive pulmonary disease [25]. Phenols are highly effective anticoagulants, antioxidants, immune enhancers, hormone modulators and they modify the prostaglandin pathways, protect platelets from clumping, and inhibits the enzymes which stimulate the inflammation. Phenols can help manage blood pressure levels and keep blood vessels healthy and flexible. promoting good circulation. They also help reduce chronic inflammation, another risk factor for heart disease. Phenols can reduce and help control blood sugar levels [26]. The terpenoids occur when the olfactory system is tied to emotional centers in the brain, so inhaling specific terpenoids can positively affect mood and an

overall sense of well-being. Certain other terpenoids have been shown to have neurotherapeutic, anti-inflammatory, antioxidant, and pain-relieving benefits [27].

**TABLE 1A-B: QUALITATIVE ESTIMATION OF PHYTOCHEMICALS**

| S. no. | Name of the Compound | Observation |
|---|---|---|
| 1 | Alkaloids | ++ |
| 2 | Flavonoids | +++ |
| 3 | Phenols | +++ |
| 4 | Terpenoids | ++ |
| 5 | Saponins | ++ |
| 6 | Tannins | + |
| 7 | Phlobatannins | + |
| 8 | Coumarins | + |
| 9 | Emodin | + |
| 10 | Anthocynanins | ++ |
| 11 | Lekoanthocyanin | + |
| 12 | Anthroquinone | + |
| 13 | Steroid | + |
| 14 | Carbohydrates | ++ |
| 15 | Proteins | +++ |
| 16 | Glycosides | + |
| 17 | Xanthoproteins | ++ |
| 18 | Cardiac glycosides | ++ |

| S. no. | Name of the Compound | Observation |
|---|---|---|
| 1 | Alkaloids | 0.86 |
| 2 | Flavonoids | 2.36 |
| | Tannin | 0.35 |
| 4 | Lignin | 0.42 |
| 5 | Glycosides | 0.06 |
| 6 | Serpentines | 0.13 |

Saponins decrease blood lipids, lower cancer risk, and lower blood glucose response. A high saponin diet can be used to inhibit dental caries and platelet aggregation, treat hypercalciuria in humans, and as an antidote against acute lead poisoning. Saponins possess antioxidant effects on the skin and protect it against UV damage *via* inhibiting extracellular matrix degradation and anti-irritation. Saponins found in some plants can be alternative to antibiotics, with anti-microbial activities. The main role of saponins is antibiofilm activity which acts against biofilm of disease-causing pathogens [28]. Tannins are antioxidants that fight against inflammation and have anti-bacterial effects. They may protect against heart disease and contain potentially cancer-preventing properties. Tannins are a group of polyphenolic compounds widely present in the plant region and possess various biological activities, including anti-microbial, anti-parasitic, antiviral, antioxidant, anti-inflammatory, and immunomodulation [29]. Phlobatannins have been reported for its wound healing properties; these are anti-inflammatory, analgesic, antioxidant, and reducing sugar [30]. Coumarins have antithrombotic, anti-inflammatory, and are used as oral coagulant and rodenticide. Coumarins may also have antiviral effects and are highly toxic in rodents. Some of the coumarins compounds have anti-microbial effects, such as an inhibitory effect against C. In addition, coumarins can induce macrophages, which negatively affect bacterial infections. Coumarins exhibit an inhibitory role against various viruses such as HIV, Influenza, Enterovirus 71 (EV71), and coxsackievirus A16 (CVA16) [31]. Emodin has an antiangiogenetic effect in pharmaceutical studies, indicating that emodins have anti-inflammatory, anti-cancer, and anti-microbial activity. Emodin binds to redox-active enzymes, and its effectiveness depends on the oxidative and respiratory status of cells [32].

Emodin, also known as 1,3,8-trihydroxy-6-methyl anthraquinone, is one of the agents extracted from several herbaceous plants. It possesses anti-inflammatory, anti-cancer, and antifibrotic effects [33]. Anthocyanins have many effects on protecting cells, tissues and vital organs. Cardiovascular disease and risk factors, such as high blood pressure and hardening of the arteries, Cancer, Impaired immune function, Diabetes, Neurological disorders, such as Alzheimer's disease and dementia, Symptoms of poor cognitive function, including poor memory and trouble concentrating, Fatigue, Poor recovery from exercise/physical activity, Vision loss, Obesity.

Anthocyanins, the type of flavonoid, have been detected in lung tissue shortly after being ingested and in animal models of chronic obstructive pulmonary disease (COPD). The plant chemicals appear to reduce mucus and inflammatory secretions [34]. Leucoanthocyanins are potent antioxidant and increase the bone and elasticity of capillary walks. Making them more resistant to hemorrhages, ruptures and infections. Capillary strength also results from the antioxidant free radical scavenging of the lekoanthocynins. Anthraquinones are potent laxatives and can be irritating to both the upper and lower parts of the gastrointestinal tract. Anthraquinone has anti-microbial and anti-inflammatory agents. Steroids are used to increase muscle tissue due to enhanced

protein synthesis. Decreased body fat percentage. Increased muscle strength and power. Enhanced recovery from workouts and injury. Improved bone mineral density. Better muscle endurance and increased red blood cell production. Mitochondrial respiration is also indicated as a crucial factor in the effectiveness of some anti-cancer drugs [35]. Steroids have a placssible mechanisms of action of reducing the severity of lung disease in acute respiratory distress syndrome (ARDS). Many of the large randomized controlled trials in adults that have shaped the way ARDS is treated today have excluded patients with severe chronic respiratory disease [36].

Carbohydrates are body's main source of energy. They help fuel the brain, kidneys, heart muscles, and central nervous system. Fiber is a carbohydrate that aids in digestion, helps you feel full, and keeps blood cholesterol levels in check. Energy for the body uplifts mood, helps to sleep better, provides fiber to the body, helps to prevent diseases, and prevents blood clots. Useful to control weight, improve the digestive system, helps to keep memory sharp, best nutrient for athloes, risk of cancer is reduced with carbohydrates and healthy skin. Carbohydrates use more oxygen and produce carbon dioxide. Whereas fat produces less carbon dioxide for the amount of oxygen consumed. The respiratory quotient is defined as the value of carbon dioxide released to the volume of oxygen consumed during the respiration process [37].

Protein is also a critical part of the processes that fuel energy and carry oxygen throughout the body in the blood. It also helps make antibodies that fight off infections and illnesses and helps keep cells healthy and create new ones. Protein its used for reduces appetite and hunger levels. Increases muscle mass and strength.

Good for bones. Reduces cravings and desire for Late-Night snacking. Boosts metabolism and increases fat burning. Lower blood pressure. Helps maintain weight loss and Does not harm healthy kidneys. Respiratory proteins enhance the capacity of the blood for oxygen transport and support intracellular storage and delivery of oxygen. Hemoglobin and hemocyanin are the respiratory proteins that occur in the pancrustaca [38]. Glycosides show anti-microbial activity; glycosides are cardioprotective and used to treat the cardiac arrhythmia and congestive heart failure.

**Qualitative and Estimation of Minerals:** Spices mixture was subjected to mineral analysis in **Table 2A** & **B**. The minerals such as Iron, Sulphur, Manganese, Sodium, Organic carbon, and Zinc were abundantly present in the spices mixture extract. Copper and Phosphorus were moderately present. Calcium, Potassium, Magnesium, Nitrogen, Boron, Selenium, Molybdinum were present in trace amounts in the mixture extract. Iron is a mineral vital to the proper function of haemoglobin, a protein needed to transport oxygen in the blood.

Iron also has a role in various other important processes in the body. Iron helps to preserve many vital functions in the body, including general energy and focus, gastrointestinal processes, the immune system, and the regulation of body temperature (Judith Marcin *et al.,* 2018) [39]. Calcium is used to help bone and teeth formation, Help maintain body strength, Assist in the movement of muscles, Assist with nerve messaging between the brain and body, Help blood flow as vessels relax and constrict, Release hormones and enzymes that help with body functions [40]. Potassium helps to regulate fluid balance, muscle contraction and nerve signals. A high potassium diet may help reduce blood pressure and water retention, protect against stroke and prevent osteoporosis and kidney stones.

They assist in essential body functions, including digestion, heart rhythm, and $P^H$ balance [41]. Magnesium is the fourth most abundant mineral in the human body. It is involved in hundreds of biochemical reactions in the body. It fights against depression and type 2 diabetes. Magnesium can lower blood pressure and it has anti-inflammatory. It can help to prevent migraines and it reduces insulin resistance. Magnesium improves PMS (premenstrual syndrome) Symptoms. Magnesium plays a role in over 300 enzyme reactions in the human body. It's functions may include helping with muscle and nerve function and supporting the immune system [42]. Nitrogen reactions with haemoglobin in blood, causing the oxygen-carrying capacity of the blood to decrease. Decrease functioning of the thyroid gland. Vitamin A

shortage. Fashioning of nitro amines is known as one of the most common causes of cancer.

**TABLE 2 A-B: QUALITATIVE & ESTIMATION OF MINERALS**

| S. no. | Name of the parameter | Quantity |
|---|---|---|
| 1. | Total iron (ppm) | +++ |
| 2. | Total Calcium (%) | + |
| 3. | Total Potassium (%) | + |
| 4. | Total Magnesium (%) | + |
| 5. | Total Nitrogen (%) | + |
| 6. | Total Sulphur (%) | +++ |
| 7. | Total Manganese (ppm) | +++ |
| 8. | Total sodium (%) | +++ |
| 9. | Organic carbon (%) | +++ |
| 10. | Total Zinc (ppm) | +++ |
| 11. | Total Phosphorus (%) | ++ |
| 12. | Total Copper (ppm) | ++ |
| 13. | Total Boron (ppm) | + |
| 14. | Total Selenium (ppm) | + |
| 15. | Total Molybdenum (ppm) | + |

| S. no. | Name of the parameter | Quantity |
|---|---|---|
| 1. | Ash (%) | 0.32 |
| 2. | Total Iron (ppm) | 84.23 |
| 3. | Total Calcium (%) | 2.62 |
| 4. | Total Potassium (%) | 2.36 |
| 5. | Total Magnesium (%) | 1.26 |
| 6. | Total Nitrogen (%) | 1.05 |
| 7. | Total Sulphur (%) | 0.69 |
| 8. | Total Manganese (ppm) | 0.56 |
| 9. | Total Sodium (%) | 0.54 |
| 10. | Organic carbon (%) | 0.52 |
| 11. | Total Zinc (ppm) | 0.42 |
| 12. | Total Phosphorus (%) | 0.15 |
| 13. | Total Copper (ppm) | 0.11 |
| 14. | Total Boron (ppm) | 0.02 |
| 15. | Total Selenium (ppm) | 0.01 |
| 16. | Total Molybdenum (ppm) | 0.01 |

The body needs sulphur to build and fix DNA and protect cell from damage that can lead to serious disease. Sulphur also assists in metabolizing food and contributes to the health of the skin, tendons and ligaments. The two amino acids sulphur are methionine and cysteine. Sulphur is mainly used to joint and muscle pain, allergies, dandruff [43]. Manganese helps to body from connective tissue, bones, blood-clotting factors and sex hormones. It also plays a role in fat and carbohydrates metabolism, calcium absorption, and regulating blood sugar. Manganese is also necessary for normal brain and nerve function. It may improve bone health with other nutrients. Have strong antioxidants properties, helps to reduce inflammation, contributes to good thyroid health and aid wound healing [44]. The body uses sodium to control blood volume. And it also needs muscle and nerve work properties. It regulates fluids levels, and it prevents sunstroke and stroke also. It relieves muscle cramps and skincare. It eliminates carbon dioxide and controls glucose absorption, then maintains acid-base balance. Reduce the risk of a heart attack and lower LDL cholesterol. Prevent congestive heart failure, and decrease the risk of kidney damage. Protect vision, and it reduces the hardening and thickening of arteries. Reduce bloating and swelling and the amount of drink. Lower risk of stomach cancer. All living organisms contain carbon, which forms the basis for all of the organic molecules in the body. Carbon is the second most abundant element in the human body, accounting for 20% of body weight [45]. Zinc is a nutrient that supports childhood and the immune system.

Low zinc levels can increase a person's risk of disease and illness. Immune function is treating diarrhea and wound healing. Chronic disease also prevents sexual health, osteoporosis, and neurological symptoms [46]. The body uses phosphorus to keep the bones strong and healthy. Phosphorus also helps remove waste and repair damaged tissues. Keeping the bone teeth strong, helping the muscles contract, aiding muscle recovery after exercise, and filtering and removing waste from the kidneys. Promoting healthy nerve conduction throughout the body. Making DNA and RNA and managing the body's energy usage and storage [47]. Copper helps to make red blood cells and keep nerve cells healthy. Collagen is a protein that helps make up the bones and tissues.

Absorb iron into the body and turn it into sugar, skin and heart-healthy, anxiety and depression. Balance hypertension and aids the functioning of the thyroid gland. It will prevent anemia, cure arthritis and inflamed joints, negate infection, and increase brain efficiency. Copper helps generate heat in the human body, aiding communication between different cells, breaking down certain foods to become haemoglobin, and boosting metabolism [48]. Boron is used to build strong bones, treat osteoporosis, aid in building muscles and increasing testosterone levels, and improve thinking skills and muscle coordination. Mainly boron is treated for diabetes, high cholesterol, menopausal symptoms, osteoarthritis, rheumatoid

arthritis, and psoriasis. Boron used to enhance cell selectively of radiation therapy and may lead to a new generation of drugs. Selenium enhances the T-lymphocyte's immune response. Selenium causes the formation of natural killer cells. It prevents inflammation, liver necrosis, cardiac disease, asthma, viral infections, and neurological disorders and enhances fertility by increasing sperm motility [20]. Molybdenum is an important component in an enzyme involved in oxidation-reduction reactions. It also contains a substance that is of great pharmacological and biochemical value. Its antioxidant potential is high, and its consumption promotes healthy living [20].

**Gas Chromatography-Mass Spectroscopy Method for Identification of Bioactive Compounds in Spices Mixture Extract:** Forty-five compounds were identified in Spices mixture extract by GC-MS analysis. The active principles with their Retention Time (RT), Molecular Formula (MF), Molecular Weight (MW), and concentration(%) are represented in **Fig. 1** and **Table 3.** The most prevailing compounds Piperine, Pyrrolidine,1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2, 4 - pentadienyl] -,(E,E)-,(2E,4E)-N-Isobutyloctadeca-2, 4-dienamide,(2E,4E,14E)-N-Isobutylicosa-2, 4, 14-trienamide,Piperidine, 1-[5-(1, 3-Benzodioxol-5-YL)-1-oxo-2, 4-Pentadienyl]-,(E,E)-, -(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) pent-2-en-1-one, Octadecanoic acid, cis-Vaccenic acid, 9,12-Octadecadienoic acid, n-Hexadecanoic acid, 2,4-Decadienamide, N-isobutyl-, 1-Naphthalenol,1, 2, 3, 4, 4a, 7, 8, 8a-octahydro-1, 6 – dimethyl - 4 -(1-methylethyl)-,[1R-(1.alpha., 4. beta., 4a. beta.,8a.beta.)]-. The biological activities listed in **Table 4** are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The results confirm the presence of constituents known to exhibit medicinal values and pharmacological activities.

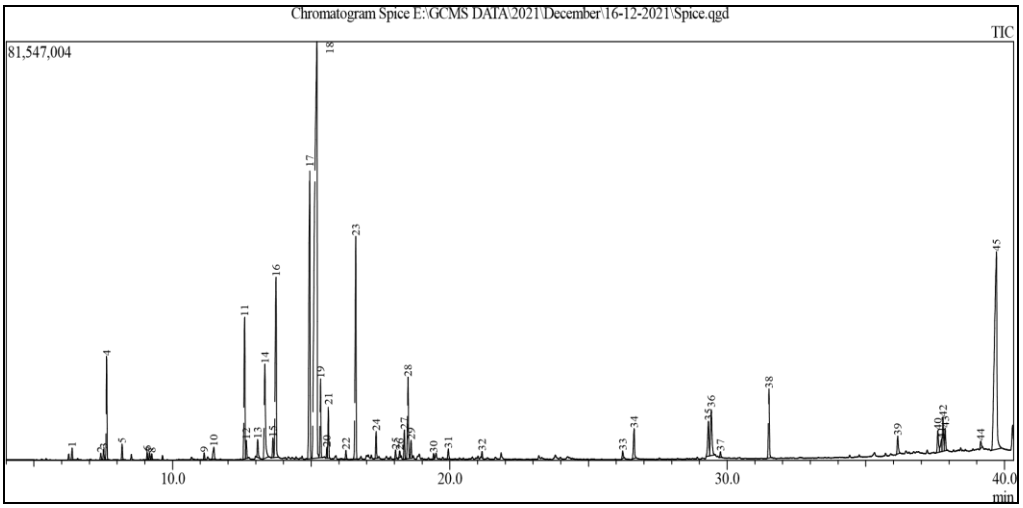


**FIG. 1: CHROMATOGRAM OBTAINED FROM THE GC-MS ANALYSIS OF SPICES MIXTURE EXTRACT**

**TABLE 3: PHYTO COMPONENTS IDENTIFIED IN SPICES MIXTURE EXTRACT BY GC-MS ANALYSIS**

| S. no. | RT | Name of the Compound | Mol. Formula | Mol.Wt | Area% |
|---|---|---|---|---|---|
| 1 | 6.377 | Bicyclo[3.1.1]Heptane, 6,6-Dimethyl-2-Methylene- | $C_{10}H_{16}$ | 136.2340 | 0.28 |
| 2 | 7.412 | Benzene, Methyl(1-Methylethyl) | $C_{13}H_20$ | 176.30 | 0.16 |
| 3 | 7.515 | D-Limonene | $C_{10}H_{16}$ | 136.23 | 0.26 |
| 4 | 7.621 | 2-Oxabicyclo[2.2.2]Octane, 1,3,3-Trimethyl | $C_{10}H_{18}O$ | 154.253 | 2.27 |
| 5 | 8.179 | 1,4-Cyclohexadiene, 1-Methyl-4-(1-Methylethyl)- | $C_7H_{10}$ | 94.15 | 0.37 |
| 6 | 9.083 | (1R,4R,5S)-1-Isopropyl-4-methoxy-4-methylbicyclo[3.1.0]hexane | $C_{11}H_{20}O$ | 168.28 | 0.18 |
| 7 | 9.168 | 1,6-Octadien-3-ol, 3,7-Dimethyl- | $C_{11}H_{18}O_2$ | 182.2594 | 0.17 |
| 8 | 9.249 | Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)- | $C_{10}H_{18}O$ | 154.2493 | 0.14 |
| 9 | 11.14 | 3-cyclohexen-1-ol, 4-Methyl-1-(1-Methylethyl)- | $C_{10}H_{18}O$ | 154.2493 | 0.16 |
| 10 | 11.488 | 3-Cyclohexene-1-Methanol, .alpha.,.alpha.,4-Trimethyl- | $C_{13}H_{22}O_2$ | 210.3126 | 0.46 |

| | | | | | |
|---|---|---|---|---|---|
| 11 | 12.598 | Propanal, 2-methyl-3-phenyl- | $C_{10}H_{12}O$ | 148.2017 | 4.06 |
| 12 | 12.661 | 1,6-Octadien-3-ol, 3,7-Dimethyl-, Acetate | $C_{12}H_{20}O_2$ | 196.2860 | 0.44 |
| 13 | 13.069 | Benzoin methyl ether | $C_{15}H_{14}O_2$ | 226.2705 | 0.54 |
| 14 | 13.328 | Cinnamaldehyde, (E)- | $C_9H_8O$ | 132.1592 | 3.49 |
| 15 | 13.62 | 4-Isopropylcyclohexa-1,3-dienecarbaldehyde | $C_{10}H_{14}O$ | 150.2176 | 0.54 |
| 16 | 13.73 | 1,4-p-Menthadien-7-al | $C_{10}H_{14}O$ | 150.2176 | 6.05 |
| 17 | 14.946 | 3-Cyclohexene-1-Methanol, .alpha.,.alpha.,4-Trimethyl-, acetate | $C_{12}H_{20}O_2$ | 196.2860 | 9.71 |
| 18 | 15.204 | Phenol, 2-Methoxy-4-(2-Propenyl)- | $C_{12}H_{14}O_3$ | 206.2378 | 30.62 |
| 19 | 15.336 | Acetophenone, 4'-(1-Hydroxy-1-Methylethyl)- | $C_8H_8O_2$ | 136.1479 | 1.9 |
| 20 | 15.56 | 2,6-Octadien-1-ol, 3,7-Dimethyl-, Acetate | $C_{12}H_{20}O_2$ | 196.2860 | 0.27 |
| 21 | 15.619 | .Alfa.-Copaene | $C_{15}H_{24}$ | 204.3511 | 1.28 |
| 22 | 16.252 | Bicyclo[7.2.0]undec-4-ene, 4,11,11-Trimethyl-8-Methylene-, (Z)-(1R,9S)-(-)- | $C_{15}H_{24}$ | 204.3511 | 0.22 |
| 23 | 16.607 | Caryophyllene | $C_{15}H_{24}$ | 204.36 | 6.55 |
| 24 | 17.34 | 1,4,8-Cycloundecatriene, 2,6,6,9-Tetramethyl-, (E,E,E)- | $C_{15}H_{24}$ | 204.3511 | 0.67 |
| 25 | 18.041 | Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]- | $C_{15}H_{24}$ | 204.3511 | 0.22 |
| 26 | 18.19 | Azulene, 1,2,3,4,5,6,7,8-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl)-, [1S-(1.alpha.,4.alpha.,7.alpha.)]- | $C_{15}H_{24}$ | 204.3511 | 0.15 |
| 27 | 18.358 | .beta.-Bisabolene | $C_{15}H_{24}$ | 204.3511 | 0.72 |
| 28 | 18.492 | Phenol, 2-methoxy-4-(2-propenyl)-, acetate | $C_{12}H_{14}O_3$ | 206.2378 | 2.35 |
| 29 | 18.6 | Naphthalene, 1,2,3,5,6,8A-Hexahydro-4,7-Dimethyl-1-(1-Methylethyl)-, (1S-cis)- | $C_{15}H_{24}$ | 204.3511 | 0.48 |
| 30 | 19.413 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)- | $C_{15}H_{26}O$ | 222.3663 | 0.15 |
| 31 | 19.944 | 5Oxatricyclo[8.2.0.0(4,6)]Dodecane,,12-Trimethyl-9-Methylene-,[1R(1R*,4R*,6R*,10S*)]- | $C_{15}H_{24}O$ | 220.3505 | 0.27 |
| 32 | 21.159 | 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1.alpha.,4.beta.,4a.beta.,8a.beta.)]- | $C_{15}H_{26}O$ | 222.3663 | 0.14 |
| 33 | 26.232 | 2,4-Decadienamide, N-isobutyl-, (E,E)- | $C_{14}H_{25}NO$ | 223.3544 | 0.22 |
| 34 | 26.644 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.4241 | 0.98 |
| 35 | 29.324 | 9,12-Octadecadienoic acid (Z,Z)- | $C_{18}H_{32}O_2$ | 280.4455 | 1.33 |
| 36 | 29.425 | cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 282.5 | 2 |
| 37 | 29.755 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.4772 | 0.16 |
| 38 | 31.507 | 12-O-Acetylingol 8-Tiglate | $C_{27}H_{38}O_8$ | 490.6 | 2 |
| 39 | 36.149 | (E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl)pent-2-en-1-one | $C_{17}H_{21}NO_3$ | 287.3535 | 0.63 |
| 40 | 37.595 | Piperine | $C_{17}H_{19}NO_3$ | 285.34 | 0.82 |
| 41 | 37.725 | Piperidine, 1-[5-(1,3-Benzodioxol-5-YL)-1-oxo-2,4-Pentadienyl]-, (E,E) | $C_{17}H_{19}NO_3$ | 285.3377 | 0.55 |
| 42 | 37.784 | (2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide | $C_{24}H_{43}NO$ | 361.6043 | 1.03 |
| 43 | 37.861 | (2E,4E)-N-Isobutyloctadeca-2,4-dienamide | $C_{22}H_{41}NO$ | 335.5670 | 0.65 |
| 44 | 39.144 | Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E,E)- | $C_{16}H_{17}NO_3$ | 271.3111 | 0.23 |
| 45 | 39.708 | Piperine | $C_{17}H_{19}NO_3$ | 285.3377 | 14.13 |

**TABLE 4: BIOLOGICAL ACTIVITIES FOR IDENTIFIED BIOACTIVE COMPONENTS BY USING DR. DUKES DATABASE**

| S. no. | Name of the Compound | Biological Activity |
|---|---|---|
| 1 | D-Limonene | Digestive, Detoxicant, Disinfectant |
| 2 | Benzoin methyl ether | Methyl-Donor |
| 3 | 1,4-p-Menthadien-7-al | Anesthetic-potentiator, Antitumor (Breast) (Lung) (Prostate), Asthma-preventive, Perfume, Phytohormone |
| 4 | 3-Cyclohexene-1-Methanol, .alpha.alpha.,4-Trimethyl-, Acetat | TNF-alpha-Inhibitor |
| 5 | 2,6-Octadien-1-ol, 3,7-Dimethyl-, Acetate | Provide Oligosaccharides |
| 6 | .beta.-Bisabolene | Beta-Andrenergic-Agents |
| 7 | n-Hexadecanoic acid | Antitumor (Nasopharynx), Nauseant, Nicotinic, Antioxidant and anti inflammatory activity |

| | | |
|---|---|---|
| 8 | cis-Vaccenic acid | Acidulant |
| 9 | Octadecanoic acid | Acidulant, Antipreventive, Flavour, Fungicide, pesticide, perfumery, Anti-inflammatory, hypocholesterolemic, Cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, anticoronary |
| 10 | 12-O-Acetylingol 8-Tiglate | Ozone-Scavenger, Suppress Osteoclast Activity |
| 11 | (2E,4E,14E)-N-Isobutylicosa-2,4,14-trienamide | Energizer,Expectorant |
| 12 | (2E,4E)-N-Isobutyloctadeca-2,4-dienamide | Energizer, Expectorant |

Biologically active compounds possess detoxicant activity, Antitumor (Breast) (Lung) (Prostate), Asthma-preventive, Antitumor (Nasopharynx), Antioxidant, Anti-inflammatory, Antipreventive, Antihistaminic, Anticoronary, Cancer preventive, Hepatoprotective, and Hypocholesterolemic [49]. They act as a TNF-alpha-Inhibitor, Anesthetic-potentiator, Nauseant, Nicotinic, Acidulant, Flavour, Fungicide, Pesticide, Perfumery, Nematicide, Insectifuge, Ozone-Scavenger, and Suppress Osteoclast Activity. Most of the biologically active compounds present in **Table 5** have been reported to possess Anti Viral and Anti cold activity [50].

**UV Visible Spectroscopic Analysis for Identification of Bonds in Spices Mixture Extract:** The UV-Visible spectra were performed to identify the compounds containing σ-bonds, π-bonds, and lone pairs of electrons, chromophores and aromatic rings. The UV-Visible absorbance spectra of ethanolic extracts of the samples were recorded in the range of 200-1100 nm. The profile showed the peaks at 279.85 and 1062.95 nm with the absorption of 3.758 and 0.190, respectively [20].

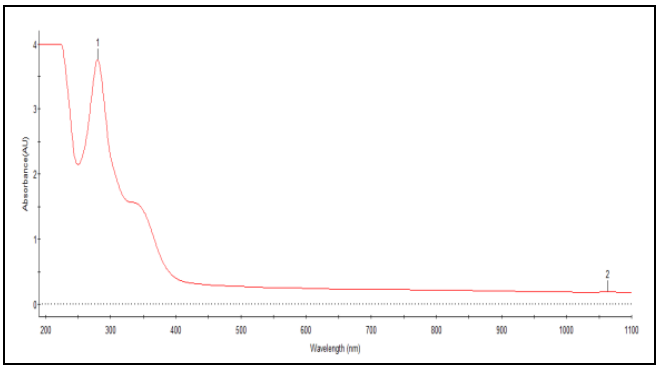


**FIG. 2: UV-VISIBLE PEAK VALUES OF SPICES MIXTURE EXTRACT**

**TABLE 5: UV-VISIBLE PEAK VALUES OF SPICES MIXTURE EXTRACT**

| S. no. | Wavelength (nm) | Absorption peak |
|---|---|---|
| 1 | 279.85 | 3.758 |
| 2 | 1062.95 | 0.190 |

The occurrence of peaks at 279.85-1062.95 nm reveals that the absorption bands are due to the presence of Flavonoids and their derivatives in the Spices Mixture extract. Thus we confirm that the spices contain the richest sources of flavonoids and their derivatives.

The flavonoids are well known for their Antioxidant Activity such as radical scavenging and metal ion chelation ability, Hepatoprotective Activity, Anti-bacterial Activity, Anti-Inflammatory Activity, Anti-cancer Activity, Anti-viral Activity, Anti-allergic Activity, Anti-adhesive Activity, Anti-convulsive Activity, Anti-cold Activity, Anti-gastric Activity and chemoprotective activity [20].

**FTIR Spectroscopic Analysis for Identification of Functional Groups in Spices Mixture Extract:** FTIR Spectrum identified the functional group of the active chemical components present in the spices based on the peak value in the infrared radiation region.

The peaks correspond to bending and stretching vibrations between the molecules of the samples. When the spice extract was passed into the FTIR, the functional group of the components was separated based on its ratio. The peak values and the functional groups were represented in **Fig. 3.** & **Table 6).**

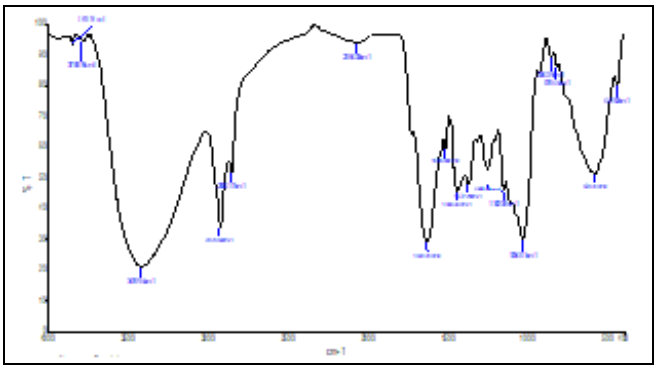

**FIG. 3: THE FTIR PEAK VALUES OF SPICES MIXTURE EXTRACT**

**TABLE 6: THE FTIR PEAK VALUES OF SPICES MIXTURE EXTRACT**

| S. no. | Peak Values | Functional groups |
|---|---|---|
| 1 | 3851.50 | Amine |
| 2 | 3786.19 | Amine |
| 3 | 3419.94 | Amine |
| 4 | 2923.63 | Alkyls |
| 5 | 2854.17 | Alkyls |
| 6 | 2064.00 | Alkynes |
| 7 | 1631.91 | Alkene |
| 8 | 1515.60 | Alkene |
| 9 | 1444.93 | Alkene |
| 10 | 1372.20 | Methyl |
| 11 | 1152.25 | Esters, Alcohols & COOH |
| 12 | 1034.81 | Cyano group |
| 13 | 854.09 | Alkane |
| 14 | 819.44 | Alkane |
| 15 | 579.31 | Halogen compound |
| 16 | 441.62 | Unknown |

The results of the FTIR confirmed the presence of Amines, Alkyls, Alkanes, Alkynes, Alkenes, Methyls, Esters, Alcohols, Carboxylic acid, Cyano group, and Halogen compounds [51].

**HPLC Analysis for Flavonoid profile in Spices Mixture Extract:** HPLC was performed to identify the spice mixture extract's flavonoids and displayed in **Fig. 4** & **Table 7**. The result confirms the presence of various flavonoids like Quercetin, Rutin, Catechin, and Gallic acid in Spices Mixture extract. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants [52].

Flavonoids are found in many different species of plants and can be subdivided into flavonols, flavones, flavanols, flavanones, anthocyanidins, proanthocyanidins, and isoflavones, each with their own individual compound [53]. Flavonoids are proposed to reduce the incidence of respiratory infections because they have a range of physiologic effects in humans, including antiviral, anti-inflammatory, cytotoxic, anti-microbial, and antioxidant [54].

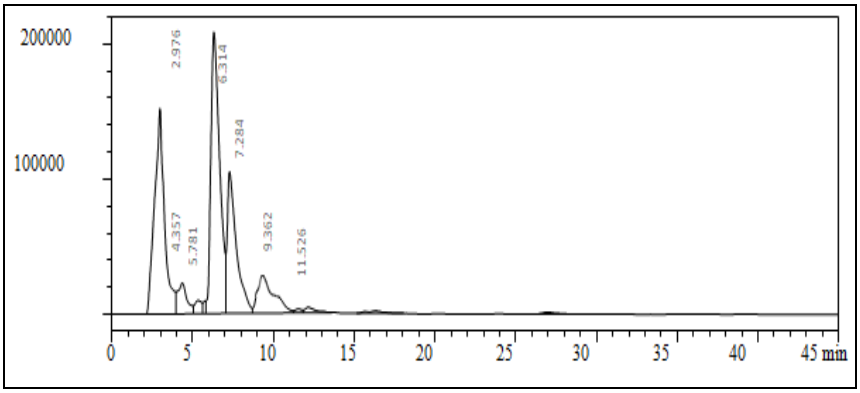


**FIG. 4: CHROMOTAGRAM OBTAINED FROM HPLC ANALYSIS OF SPICES MIXTURE EXTRACT**

**TABLE 7: COMPOUNDS IDENTIFIED BY HPLC ANALYSIS**

| Peak | Retention Time | Area | Height | Area% | Height % | Comopunds identified by literature |
|---|---|---|---|---|---|---|
| 1 | 2.976 | 6466236 | 152203 | 29.106 | 27.859 | Gallic acid |
| 2 | 4.357 | 917077 | 22835 | 4.128 | 4.180 | Unknown |
| 3 | 5.342 | 294425 | 9802 | 1.325 | 1.794 | Unknown |
| 4 | 5.781 | 106696 | 9470 | 0.480 | 1.733 | Unknown |
| 5 | 6.314 | 7872396 | 208318 | 35.435 | 38.131 | Catechin |
| 6 | 7.284 | 4183442 | 104883 | 18.830 | 19.198 | Violaxanthin |
| 7 | 9.362 | 1909943 | 27750 | 8.597 | 5.079 | Unknown |
| 8 | 11.526 | 82011 | 2847 | 0.369 | 0.521 | Astaxanthin |
| 9 | 12.152 | 178547 | 3926 | 0.804 | 0.719 | Rutin |
| 10 | 15.662 | 32932 | 1178 | 0.148 | 0.216 | Rutin |
| 11 | 16.318 | 93580 | 1790 | 0.421 | 0.328 | P. Caumaric acid |
| 12 | 26.971 | 79212 | 1323 | 0.357 | 0.242 | Quercetin |
| Total | | 22216495 | 546325 | 100.000 | 100.000 | |

Quercetin has a good inhibitory effect on the growth of pathogenic bacteria such as *Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus, Escherichia coli, Proteus* and *Aspergillus flavus* [55]. Quercetin has been shown to exert strong anti-pathogenic effects against several causal agents of URTI in culture studies [56].

Rutin inhibits the proliferation of breast, colon, lung, and prostate cancers and other tumors. Rutin possesses anti-inflammatory, anticarcinogenic, neuroprotection, antiproliferative, antimetastatic, and antioxidative stress [57]. Rutin significantly inhibited specific airway resistance and immediate-phase response and reticence of histamine, phospholipase A2, and eosinophil peroxidase. There was reduced conscription of neutrophils and eosinophils into the lung [58].

The use of rutin was also suggested in whooping cough along with vitamins C and K.Regular administrations of catechins cause beneficial effects against chronic pulmonary disorder and asthma. Catechin possesses antitumor, antioxidant, anti-inflammatory, anti-microbial, antiviral, antidiabetic, anti-obesity, and hypotensive effects [59].

The edible uses of gallic acid and its ester derivatives as flavoring agents and preservatives in the food industry, there are diverse scientific reports on biological and pharmacological activities of these phytochemicals, with emphasis on antioxidant, anti-microbial, anti-inflammatory, anti-cancer, cardioprotective, gastroprotective, and neuroprotective effects [60].

**HPTLC Analysis of Spices Mixture Extract:** HPTLC Analysis of methanolic extract of spices was carried out along with the standard flavonoids Quercetin and Rutin. Blue coloured zone at visible mode was present in the track; it was observed from the chromatogram after derivatization, confirming Quercetin and Rutin's presence in the spices extract.

The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3. The standard quercetin and rutin have RF values of 0.09 and 0.68 and the sample RF shows 0.12 and 0.71. By comparing the area of sample and standard, calculated the percentage of Quercetin and Rutin in a sample using the formula. Spices extract contains 93.53% of Quercetin and 95.23% of rutin.

Area of Standard = Percentage of Quercetin / Area of Sample extract × 100

Percentage of Quercetin& Rutin = 93.53% & 95.23%

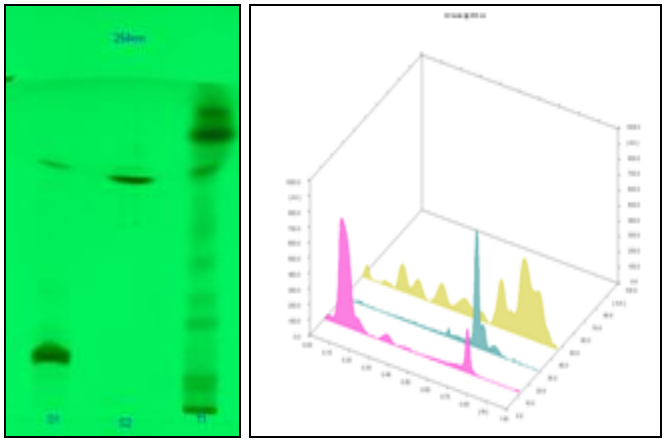

**FIG. 5: PHOTO DOCUMENTATION UNDER UV3D DISPLAY AT 254nm**

HPTLC method deals with qualitative and quantitative analytical applications such as herbal and dietary supplements, nutraceuticals, and various types of medicines. It is used in quality control and purity checks, detecting and identifying pharmaceutical raw materials, drugs, and their metabolites in biological media. HPTLC method is also a potent tool for identifying the presence of adulterants in herbal products based on the characteristic image produced and much useful for determining the presence and the quantification of both inadvertent substitution and intentional adulteration of prescription drugs [61].

Quercetin, a plant flavonoid, is a potent antioxidant and anti-inflammatory agent. Quercetin has a good inhibitory effect on the growth of pathogenic bacteria such as *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus*, and *Aspergillus flavus* [62]. Quercetin has been shown to exert strong anti-pathogenic effects against several causal agents of URTI in culture studies (56).Quercetin treatment decreases chemokine and cytokine levels in elastase. Rutin is a unique antioxidant flavonoid that is mainly found in fruit, vegetables, cereals and many other plant-based human diets. Rutin is demonstrated to inhibit the proliferation of breast, colon, lung, and prostate cancers and other tumors. Rutin possesses anti-inflammatory, anti-carcinogenic, neuroprotection, antiproliferative, antimetastatic, and antioxidative stress [57]. Rutin significantly inhibited specific airway resistance and immediate-phase response and reticence of histamine, phospholipase A2, and eosinophil peroxidase. There was reduced conscription of

neutrophils and eosinophils into the lung [58]. The use of rutin was also suggested in whooping cough along with vitamins C and K.

In cats and whippets, rutin has been effectively used in the management of idiopathic chylothorax [63].

***In-vitro* Antioxidant Activity of Spices Extract:**

**DPPH *In-vitro* Anti-oxidant Activity:** The half inhibition concentration ($IC_{50}$) of Ascorbic acid (41.28µg/ml) and extract (52.23 µg/ml) were, and respectively the extract exhibited a significant dose-dependent inhibition of DPPH assay activity. The overall present study was % of inhibitions based on the concentration of extract depended ($R^2$ = 0.9626).

DPPH radical scavenging activity of mixed spices extract, and standard ascorbic acid is presented in (3.7.1 a & b).

The DPPH was widely used for the free-radical scavenging capacity of antioxidants. DPPH showed 20µg/ml of extract has a less antioxidant capacity of 33.14±0.71 when compared to 35.69±0.53. 40µg/ml of extract has the less antioxidant capacity of 41.71±0.96 when compared to 47.08±0.81. 60µg/ml of extract has less antioxidant capacity than 58.28±1.28 when compared to 63.91±1.15. 80µg/ml of extract has less than 65.42±1.31 when compared to 78.58±1.39. 100µg/ml of extract of less antioxidant capacity 70.57±2.05 when compared to 94.62±2.53. $IC_{50}$ µg/ml of extract has more antioxidant capacity, 52.23 when compared to 41.28. $R^2$ of extract has less antioxidant capacity 0.9626 when compared to 0.9968.

**TABLE 8: GRAPHICAL REPRESENTATION OF DPPH *IN-VITRO* ANTIOXIDANT ACTIVITY**

| Concentrations (µg/ml) | % of inhibitions | |
|---|---|---|
| | Sample | Std. (Ascorbic acid) |
| 20 | 33.14±0.71 | 35.69±0.53 |
| 40 | 41.71±0.96 | 47.08±0.81 |
| 60 | 58.28±1.28 | 63.91±1.15 |
| 80 | 65.42±1.31 | 78.58±1.39 |
| 100 | 70.57±2.05 | 94.62±2.53 |
| $IC_{50}$ (µg/ml) | 52.23 | 41.28 |
| $R^2$ | 0.9626 | 0.9968 |

**Determination of Total Antioxidant Capacity:** The half inhibition concentration (IC50) of Ascorbic acid (39.04µg/ml) and extract (43.56µg/ml) were and respectively, the extract exhibited significant dose-dependent inhibition of TAA assay activity. The present overall study was % of inhibitions based on the concentration of extract depended (R2 = 0.9615).

The total Antioxidant Activity of the ethanol extract of the spices mixture is given in (3.2. a & b). The TAA is generally based on the inhibition of certain reactions in the presence of antioxidant. The TAA showed 20µg/ml of extract has less antioxidant capacity of 29.41±0.97 when compared to 34.79±0.61. 40µg/ml of extract has less antioxidant capacity of 49.67±1.04 when compared to 50.44±0.86. 60µg/ml of extract has less antioxidant capacity 66.33±1.29 when compared to 67.71±1.35. 80µg/ml of extract has less 76.47±1.37 when compared to 81.35±1.82. 100µg/ml of extract of less antioxidant capacity 83.98±2.45 when compared to 95.29±2.09. $IC_{50}$ µg/ml of extract has more antioxidant capacity 43.56 when compared to 39.04. R2 of extract has less antioxidant capacity 0.9615 when compared to 0.9979.

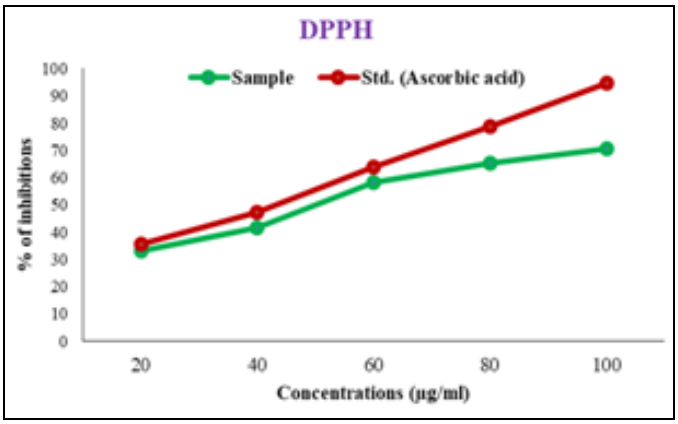


**FIG. 6: GRAPHICAL REPRESENTATION OF DPPH *IN-VITRO* ANTIOXIDANT ACTIVITY**

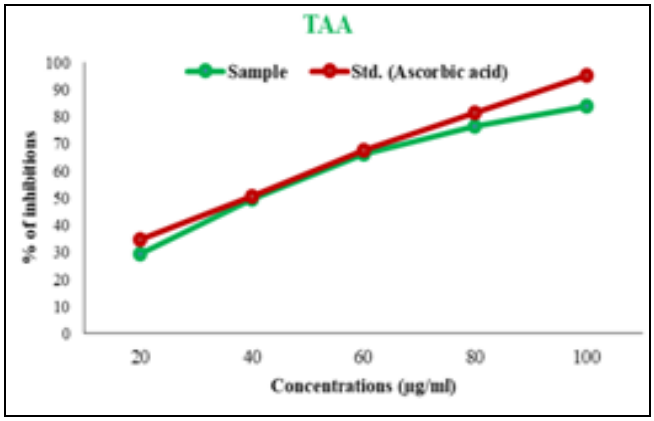


**FIG. 7: GRAPHICAL REPRESENTATION *IN-VITRO* TOTAL ANTIOXIDANT ACTIVITY**

**TABLE 9: GRAPHICAL REPRESENTATION *IN-VITRO* TOTAL ANTIOXIDANT ACTIVITY**

| Concentrations (µg/ml) | % of inhibitions | |
|---|---|---|
| | Sample | Std. (Ascorbic acid) |
| 20 | 29.41±0.97 | 34.79±0.61 |
| 40 | 49.67±1.04 | 50.44±0.86 |
| 60 | 66.33±1.29 | 67.71±1.35 |
| 80 | 76.47±1.37 | 81.35±1.82 |
| 100 | 83.98±2.45 | 95.29±2.09 |
| $IC_{50}$ (µg/ml) | 43.56 | 39.04 |
| $R^2$ | 0.9615 | 0.9979 |

**CONCLUSION:** Spices are naturally available medicines without any side effects. The abundant phytochemicals present in the spices were Flavonoids, Phenols, and Proteins. In contrast, Alkaloids, Terpenoids, Saponin, Anthocyanins, Carbohydrates, Xanthoproteins Cardiac glycosides were present in trace amounts, and other phytochemicals such as Tannins, Phlobatannins, Caumarins, Emodin, Leukoanthocyanins, Anthroquinones, Steroids, and Glycosides were slightly present. Other than phytochemicals, spices were also rich in minerals such as Iron, Sulphur, Manganese, Sodium, Carbon, and Zinc, whereas Phosphorus and Copper were present in trace amounts and other Calcium, Potassium, Magnesium, Nitrogen, Boron, and Selenium were slightly present.

Phytochemicals and Minerals have inhibitory effects against respiratory infections. GCMS was carried out to determine the biological activity of the compounds present in the sample. Twelve compounds were biologically active and mostly possess Antitumor effects in lung cancer, Antiinflammatory, Antioxidant, and especially Anti cold activity. In UV Visible spectroscopy, peaks at 279.85-1062.95 nm reveal that the absorption bands are due to the presence of flavonoids and their derivatives in the Spices Mixture extract.

Thus, we confirm that the spices contain the richest flavonoids and their derivatives sources. The flavonoids are well known for their Antioxidant activity such as radical scavenging and metal ion chelation ability, Hepatoprotective activity, Anti-bacterial activity, Anti-Inflammatory activity, Anti-cancer, Anti-viral activity, Anti-allergic activity, Anti-adhesive activity, Anti-convulsive activity, Anti-cold activity, Anti-gastric activity, and Chemopreventive Activity. The results of the FTIR confirmed the presence of Amines, Alkyls, Alkanes, Alkynes, Alkenes, Methyls, Esters, Alcohols, Carboxylic acid, Cyano group, and Halogen compounds. So, that we concluded that functional groups of chemical components present in the Spices are biologically active which regulates various physiological and biochemical activities. HPLC was performed to identify the flavonoids present in the Spices mixture extract. The result confirms the presence of various flavonoids like Quercetin, Rutin, Catechin, and Gallic acid in Spices Mixture extract. HPTLC Analysis of methanolic extract of spices was carried out along with the standard flavonoids Quercetin and Rutin. Blue coloured zone at visible mode was present in the track; it was observed from the chromatogram after derivatization, confirming Quercetin and Rutin's presence in the spice extract. Therefore the ethanolic extract of spices mixture extract contains strong bioactive compounds, particularly flavonoids that possess antioxidant and anti-respiratory activity.

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**CONFLICT OF INTEREST:** There is no conflict of interest.

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