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**FORMULATION OF HERBAL PERFUME AND IT'S ASSESSMENT  
FOR ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY BY  
EXTRACTION OF ESSENTIAL OILS**

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**ABSTRACT**

The traditional assumption that perfume are utilized uniquely for veiling the personal stench was questioned by the consequence of ongoing examination. In spite of the fact that the biological role of human body odour is very important, it likely that Perfume are not in conflict with the body odour but there is so many medicinal purposes like antioxidant, antibacterial, antiseptic and antifungal activities are present in all Aromatic plants (Volatile Oils). The extracted essential oil were further mixed with ethanol and subjected them for preparation of three different formulations. Clove, Rose, Cinnamon, Orange, Anise and Lemon oils, Glycerine, Water, Ethyl alcohol is used for making the herbal perfume. Among three formulation, Formulation C ( Anise oil, Clove oil, Cinnamon oil, Rose oil, Lemon oil, Orange oil, Glycerine, Water, Ethyl alcohol ) was shown better antibacterial activity. Here different concentrations of formulations were made and subjected for antioxidant movement i.e hydrogen peroxide scavenging activity. Among all, 0.5 µg /ml concentration of formulation C was found to be best antioxidant activity. The result obtained suggests that presence of terpenes in these essential oils is liable for antibacterial and antioxidant activity.

**Keywords: Herbal Perfume, Aroma, Refresh, Cosmetics, Antioxidant, Antibacterial**

**INTRODUCTION**

The word perfume is utilized today portray scented combinations and got from the Latin word, “per-fumus” which means

through *smoke*. The word perfumery alludes to the craft of making aromas. Perfume was further refined by the Romans, the Persians

and the Arabs. In spite of the fact that aroma and perfumery also existed in East Asia, a lot of its aromas were incense based. The fundamental fixings and strategies for making aromas are portrayed by Pliny the Elder in his *Naturalis Historia*. The world's initially recorded physicist is a lady named Tapputi an aroma creator whose presence was recorded on a 1200BcE Cuneiform tablet in Babylonian Mesopotamia. Unpredictable oils are foul and unstable results of different plant and creature species. As they have an inclination to go through vanishing on being presented to the air occasion at a surrounding temperature, they are constantly named as unpredictable oils, essential oils or ethereal oils. They generally add to the odoriferous constituents or 'embodiments' sweet-smelling plants that are utilized plentifully in upgrading the fragrance via preparing of eatables [1, 2].

These volatile oils usually structure by two modes to be specific First, by hydrolysis of certain glycosides; and Secondly, by the protoplasm directly. Volatile oils may be available specifically secretor parts, for example, in mesophyll eucalyptus leaves, sub-epidermal tissues of Lemon and Orange, in petals (Rose), in bark and leaves (Cinnamon) and in skin (Orange). The natural volatile oil and their scents had

assumed crucial part straightforwardly or in a roundabout way in people life since the start of human progress. The craft of fragrance making was first considered and utilized in the east, uniquely in India, Egypt, Persia and China. Rose flavours had been accessible in India 1000 BC and during Mughal period, oriental sort of fragrances like 'Attars' were reproduced and sent out to different nations as a traditional produce of essential oils [3, 4].

India is an emporium of aromatic and medicinal plants and has one of the most established, most extravagant and most assorted social traditions related with the utilization of sweet-smelling therapeutic plants for example scent, flavor, healing, germ-free, cell reinforcement, antibacterial, food innovation, industry insecticides, additives and alternative medication. Volatile oils are utilized in various definitions like 1) Cosmetics-Perfumes and spray, creams and powder 2) Medical-pharmaceutics preparations 3) Food Beverages, flavouring agents, additives 4) Dental preparations tooth paste, mouthwash 5) Insecticides preparations sprays, repellants etc. Essential oils/Volatile oils are separated by various methods like maceration/dissolvable, ethanolic and ecuelle extraction [5, 6].

Table 1

Pharmacological action and therapeutic uses. Therapeutic Properties	Pharmacological action	Aromatic medicinal plants
Antiseptic	To kill the pathogenic bacteria and remove the infections	Anise, lemon and rose oil
Sedative	Calming, soothing, toning, relaxing	Rose
Stomachic	Digestives	Anise and Clove
Cardiac	Heart toners,	Anise and Cinnamon
Hypnotic	Sleeping agents,	Orange and Rose
Antidiuretic	Bladder and kidney remedies	Anise and lemon
Anthelmintic	Against parasites worms	Cinnamon ,clove and Lemon
Analysis	Pain killers	Cinnamon, clove, Lemon, mint and pepper
Anti-rheumatic	Rheumatic cure	Lemon and mint
Ant gripe	Cold remedies	Pepper
Bronchodilator	Cough and Bronchitis treatment	Anise, lemon and pine
Bio stimulant	Stimulating life processes and improving resistance to infections, cancer protection	Cinnamon and Peppermint
Antiseptic	To kill the pathogenic bacteria and remove the infections	Anise
Sedative	Calming, soothing, toning, relaxing	Lemon and Peppermint

### Fragrance

The essential oils have been known for their fragrance and curative impacts on the body&soul. These aroma molecules of the oil contain numerous and intense organic plant chemicals, which are natural and exceptionally powerful medication, profiting from numerous points of view the strength of our body, psyche and spirits [7, 8].

### Antibacterial

The essential oil obtained from thyme, cinnamon, clove, lavender and, have germ-free movement against different pathogenic organisms; in any event, for those, which are accounted for to be enrolled to be safe against specific anti-toxins and parasites which are liable for mycosis and furthermore against yeast (candida).The plantsegments like citral having antiseptic activities many times more than phenol.

Antibacterial properties of essential oil play important role in their topical application on cuts, burn and wound and joint pains and sprains [9].

### Antioxidant

Due to their proven anti-aging and anti-oxidant properties, essential oils discover use in reviving and therapeutic preparation having cosmetic and curative worth. The aroma therapy has now assumed an important position in the holistic approach towards better wellbeing and cure (stresses, depression and psychosomatic disorder) [10].

### Types of Perfume [11, 12]:-

- 1) Natural Origin : Essential oil, concretes, absolutes, CO<sub>2</sub>s, tinctures, infusions.
- 2) Synthetic Origin : Primarily synthetic aromachemicals, minimal essential oils and absolutes.

**Different fragrance in perfumes:**

1) Floral 2) Citrus 3) Fruity 4) Green 5) Oceanic 6) Oriental 7) Spicy 8) Woody (Chypre)

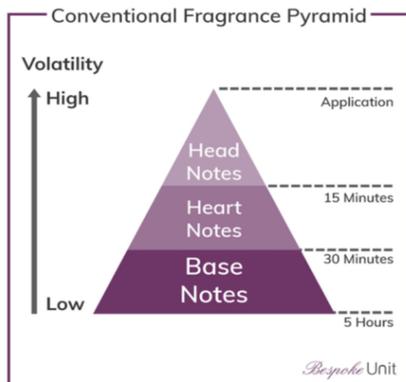


Figure 1: Fragrance Pyramid

**MATERIALS & METHODS**

All ingredients were collected during January 2019 from the Pharmacognosy lab of Ashokrao Mane College of Pharmacy, Peth Vadgaon, Maharashtra, India. The all ingredients were identified by Dr. A. U. Arvindekar, Head of Department of Pharmacognosy, Ashokrao Mane College of Pharmacy, Pethvadgaon, Maharashtra, India.

**I) MATERIALS [13, 14, 15]****Types of perfume ingredients:-**

- 1) Odorants :- 1) Concrete 2) Absolutes 3) Tinctures 4) Resinoids
- 2) Fixatives:- Natural fixatives are resinoids 1) Benzoin 2) Myrrh
- 3) Extenders :- Petroleum jelly
- 4) Blenders:- 1) Linalool 2) Hydroxycitronellal

**II) METHODS:-****A) EXTRACTION OF ESSENTIAL OILS:-**

Following methods were utilized for the extraction of essential oils.

**a) Maceration/ Solvent extraction method:-**

The most used and economically important technique for extracting aromatics in the modern perfume industry. Raw material are submerged in a solvent that can dissolve the desired aromatic compounds. Maceration lasts anywhere from hours to months. Fragrant compounds for woody and fibrous plant materials are often obtained in this manner as are all aromatics from animal sources. The technique can also be used to extract odorants that are too volatile for distillation or easily denatured by heat. Commonly used solvents for maceration/solvent extraction include ethane, hexane, and dimethyl ether. The product of this process is called a "Concrete" [16].

• **Ethanol Extaction:-** A type of solvent extraction used to extract fragrant compounds directly from dry raw materials, as well as the impure oily compounds materials resulting from solvent extraction or enfleurage. Ethanol extraction from fresh plant materials contain large quantities of water, which will also be extracted into the ethanol [16].

**b) Ecuelle extraction method:-**

It is used to obtain citrus oil. In this method, the fruit rolled over the trough lined with sharp projections. These

projections penetrate the epidermis and puncture the oil glands located in the outer portion of the peel. The fruit is then pressed to remove oil from glands, it is then sprayed with water that washes oil from the mashed peel. The resulting oil water emulsion is separated by centrifugation [16].

### B) METHOD OF FORMULATION:–

Perfume is an intricate blended of essential oil in appropriate proportion. The percent is due to the 15-20% of aromatic component in an alcohol solution 80% dilute the ingredients (oils) and carries the perfume by evaporating. All essential oils are soluble in ethyl alcohol [17] (Table 3).

Table 2

Sr.No	NAME OF INGREDIENTS	SYNONYM	BIOLOGICAL SOURCE	CHEMICAL CONSTITUENTS
1.	ANISE	Pimpinella aniseed	Dried ripe fruits of <i>pimpinellaanisumlinn.</i> Family: Umbeliferae.	Cuminic alcohol, beta – pinene, cumnaldehyde.
2.	CLOVE	Clove bud	Dried flower buds of <i>Eugenia caryophyllus</i>	Eugenol, caryophyllene, resin, tannin, alcoholic, ketone, Eugenin, ester. Family: Myrtaceae
3.	ORANGE	Apricot, citrus fruit	Obtained from the fresh orange peels of <i>citrus aurantium</i> Family : Rutaceae	D-linalool, limonene, pinene.
4.	LEMON	lemon fruit	Obtained by expression method from fresh peel of the ripe fruits of <i>citrus limonis</i> Family : Rutaceae	Limonene, linalool.
5.	CINNAMON	Cinnamon bark	Obtained from Dried inner bark of the shoots of coppiced trees of <i>Cinnamomum zeylanicum</i> Family: Lauraceae.	Cinnamon oil, Eugenol and other terpene.
6.	ROSE	Rosebush, wine	Obtained by steam distillation from fresh leaves and stem of <i>plergoniumgraveolance</i> Family: Geraniaceae.	Citronellol, geraniol.

Table 3: Essential oils

Sr. No.	Name of essential oil	Part of the plant where Oil is obtained
1.	Anise oil	Dried seeds
2.	Clove oil	Dried flower buds
3.	Orange oil	Dried peel of fruit
4.	Lemon oil	Dried peel of fruit
5.	Cinnamon oil	Dried bark
6.	Rose oil	Petals of flower

Table 4: Formula for 10 ml

Sr. No.	Name of oils	Formulation- A (ml)	Formulation- B (ml)	Formulation- C (ml)
1.	Anise oil	0.8	0.8	0.8
2.	Clove oil	0.4	0.4	0.6
3.	Lemon oil	0.4	0.4	0.4
4.	Cinnamon oil	0.4	0.4	0.4
5.	Rose oil	0.4	0.4	0.4
6.	Peppermint oil	-	0.4	-

7.	Orange oil	-	-	0.4
8.	Water	Q.S.	Q.S.	Q.S.
9.	Glycerine	Q.S.	Q.S.	Q.S.
10.	Ethyl Alcohol	7.6	7.2	7

- 1) Weighed accurately all the ingredients.
- 2) Bottles were sterilised and, especially if you are reusing bottles. They need to be clean and sterile.
- 3) Added the accurately weighed alcohol in the bottles
- 4) Then added determined quantity of all essential oils.
- 5) Added 2 tablespoons of distilled water/spring water. If you are making a perfume spray, and more water.
- 6) Added approximately 5 drops of glycerine, this helps preserve the fragrance.
- 7) Pour the perfume into your bottle, use a funnel if you need to. Store in a cool and dry place and protect from light to for long lasting fragrance.

### C) METHODS OF EVALUATION [18, 19]:-

#### 1) Organoleptic evaluation:-

Colour and odour. This evaluation is done by using a sensory organs.

#### 2) Microbiological evaluation:- Antibacterial activity.

##### • Diffusion method/Agar plate method/Cup plate method:-

Cup plate method is one of the official methods in IP, where the test samples

diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms is restricted entirely to a circular area or zone around the cavity containing the solution of an antibiotic substance. The antimicrobial activity is expressed as zone diameter in millimeters, which is measured by a scale.

#### Microorganisms:-

Gram-positive organisms used in the study were *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*.

#### Materials used

- 1) Nutrient agar
- 2) Sterile petri dishes
- 3) Sterile micropipette
- 4) Sterile cotton swab
- 5) Sterile cork borer
- 6) Sterile test tubes containing the solutions of the test compounds of desired concentrations.

#### Preparation of nutrient agar:-

Definite volumes of peptone (0.6%), yeast extract (0.15%) and di-potassium dihydrogen phosphate (0.36%) were dissolved in distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 minutes (Figure 2).



Figure 2: Nutrient agar

### Preparation of Agar plate:- (Figure 3)

- 1) Using heat resistant hand protection, hold the flask over the flame and stirred the mixture gently using sterile stirrer while heating.
- 2) The boiling of mixture continued for about one minute, and then removed from heat.
- 3) Melted agar poured into the petri plate to cover the bottom and replaced the lid immediately.
- 4) The agar plates allowed to cool and set. (The medium will set like gelatin at room temp).

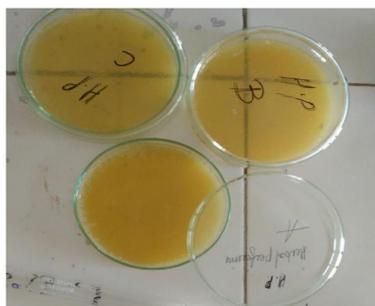


Figure 3: Nutrient Agar plate

### Cultivation of Bacteria:- (Figure 4)

- a) Using Cotton Swab :-  
Cotton swab containing gram-positive microorganisms completely inserted into the prepared area of agar plate with

twisting motion and stick the swab pressed lightly with forceps or another swab. Incubated the plates for 24 hrs at 37°C.



Figure 4: Cultivation of Bacteria

### Experimental Procedure: -

- 1) The sterile borer was used to prepare five cups of 10 mm diameter, in the medium of each Petri dish.
- 2) At the centre, one more cup was made for compared with the other cups; its zone of inhibition was measured to compared with the zone of inhibition of the sample preparation.
- 3) The prepared samples were poured like 0.2ml, 0.4ml, 0.6ml, 0.8 ml into each cup except middle cup.
- 4) All petri plates were placed into incubator for 24 hrs. at 37°C and after that measure the each zone of inhibition where bacteria was killed [20].

### 3) Antioxidant activity evaluation:-

- Hydrogen Peroxide scavenging assay:-  
The hydrogen peroxide scavenging activity was determined according to a previously described method. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Different

concentration of a sample preparation were added to hydrogen peroxide solution (0.6ml, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. The percentage scavenging of hydrogen peroxide by sample preparation and standard compound was calculated using formula:

**Scavenged activity of hydrogen peroxide (%) =  $[(A_0 - A_1) \times 100]$**

Where  $A_0$  represents the absorbance of the control and  $A_1$  represents the absorbance in the presence of the sample preparation and standard [21].



Figure 5: Dilutions of Concentrations

## RESULT AND DISCUSSION

### 1) Extraction:-

- 0.6 ml Clove oil obtained from 0.6 gm of clove powder.
- 0.8 ml of Anise oil obtained from 0.8 gm of anise powder.
- 0.4 ml of Orange oil obtained from 0.4 gm of orange powder.
- 0.4 ml Cinnamon oil obtained from 0.4 gm of cinnamon powder.
- 0.4 ml Rose oil obtained from 0.4 gm of rose powder.

f) 0.4 ml Lemon oil obtained from 0.4 gm of lemon powder.

From extraction procedure the above quantity of each powder contains 0.6ml,0.8ml,0.4ml, 0.4ml, 0.4ml, 0.4ml essential oils.

### 2) Organoleptic evaluation:- (Table 5)

### 3) Formulation:-

We used 10ml formulation to detect the antimicrobial and antioxidant activity after that activity we were selected final formulation for 50ml (Table 6).

### 4) Evaluation:-

#### a) Antimicrobial activity:-

For above antimicrobial assay using cup plate method, we prepared three agar plates A, B & C we were selected plate C because it showed greater zone of inhibition when it compared with plate A & plate B (Figure 6).

Formulation "C" was best, Hence, we had selected formulation contains in "plate C" for further antioxidants activity (Table 7).

#### b) Antioxidant Assay :- (Figure 8)

Among 3 formulation of herbal perfume, greater antibacterial activity showing formulation will be subjected for antioxidant activity.

The hydrogen peroxide scavenging activity of 0.1µg/ml of test sample was 66.66% when it compared to standard.

**% Hydrogen peroxide scavenging activity =  $\text{Test/Std.} \times 100$**

- 66.66% (0.1µg/ml)

- 2) 62.22% (0.2µg/ml)
- 3) 65.67% (0.3µg/ml)
- 4) 71.26% (0.4µg/ml)
- 5) 73.14% (0.5µg/ml)

From above observation it was concluded that 73.14% i.e. 0.5 µg/ml is showing maximum hydrogen peroxide scavenging activity.

Table 5: Organoleptic evaluation

Sr. No.	Organoleptic characters	Formulation- A	Formulation- B	Formulation- C
1.	Colour	Light Orange	Light Orange	Light Orange
2.	Odour	Slightly sweet	Slightly sweet	Slightly sweet

Table 6: For 50 ml Formula- Preparation

Sr. No.	Name of Ingredients	Quantity Taken (ml)
1)	Anise	4.0
2)	Clove	3.0
3)	Orange	2.0
4)	Lemon	2.0
5)	Cinnamon	2.0
6)	Rose	2.0
7)	Glycerine	Q.S.
8)	Water	Q.S.
9)	Ethyl alcohol	35



Figure 6: Zone of Inhibition

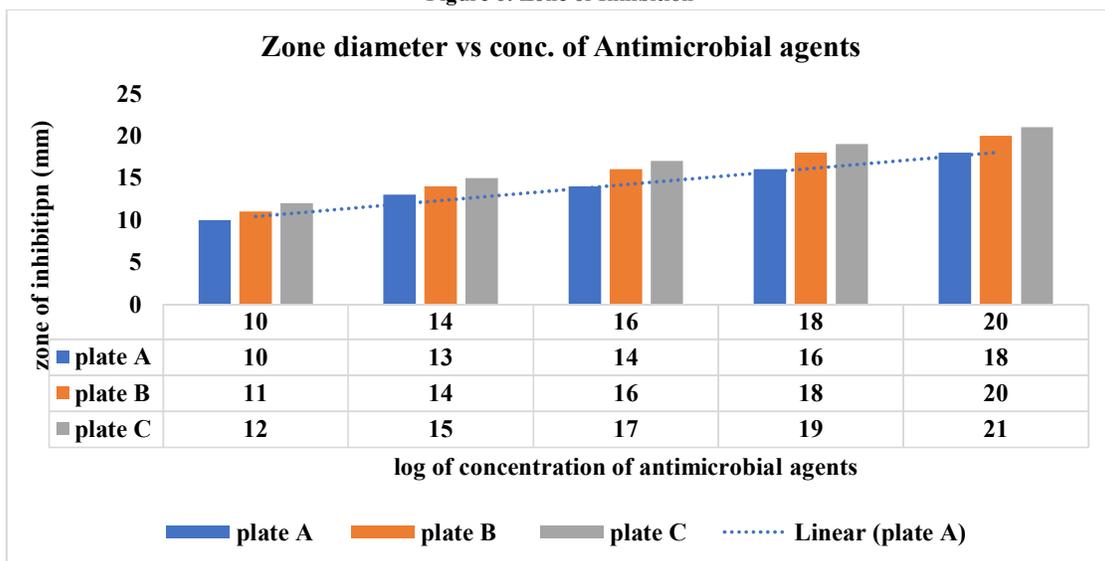


Figure 7: Zone diameter vs conc. of Antimicrobial agents

Table 7: Formulation "C" Table

Sr. No.	Name of Ingredients	Quantity Taken (ml)
1)	Anise	4.0
2)	Clove	3.0
3)	Orange	2.0
4)	Lemon	2.0
5)	Cinnamon	2.0
6)	Rose	2.0
7)	Glycerine	Q.S.
8)	Water	Q.S.
9)	Ethyl alcohol	35

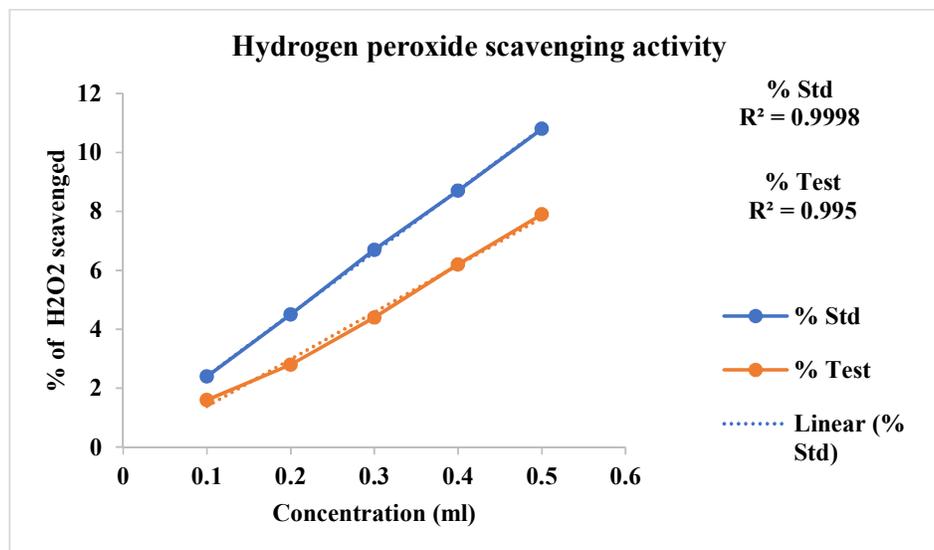


Figure 8: Hydrogen peroxide scavenging activity

## CONCLUSION

The traditional assumption that perfume are used only for masking the body odour was doubted by the result of recent research. Herbal Perfume are not in conflict with the body odour but there is so many medicinal purposes like antioxidant and antibacterial activities are present in all Aromatic plants (Volatile Oils).

The essential oils obtained from flowers, fruits, barks, leaves of the aromatic plants. There is no significant change in their activities due to terpenes are present in all aromatic plants.

All essential oils are soluble in ethanol and it gave the highest quality of Herbal perfume, hence Clove, Rose, Cinnamon, Orange, and Anise and Lemon oils for making herbal perfume. The result suggested that presence of terpenes in all essential oils have antibacterial, antiseptic, antifungal and antioxidant activities.

These oils are subjected for three formulations, which were further tested for organoleptic, antibacterial and antioxidant activity.

It was concluded that among three formulations, formulation "C" (anise oil,

cinnamon oil, lemon oil, orange oil, clove oil, rose oil and ethyl alcohol, glycerine, water) was found better antibacterial activity which was further subjected for antioxidant activity. Among different concentrations of formulation C, the 0.5µg/ml concentration shows maximum hydrogen peroxide scavenging activity i.e. 73.14%.

It was concluded that presence of terpenes in this formulation is responsible for antibacterial and antioxidant activity.

#### REFERENCES

- [1] C.K. Kokate, A.P. Purohit, S.B. Gokhale; Textbook of Pharmacognosy; Nirali Prakashan ; 48th-Edition ; January 2013.
- [2] K.R. Khandelwal; Practical Pharmacognosy techniques and experiments; Edited by Dr. Vrunda K. Sethi; Nirali Prakashan; 22<sup>nd</sup> – Edition; April 2012; Reprint February 2013.
- [3] Medicinal Plants of India, Indian Council of Medical Research, New Delhi.
- [4] Miller Phytochemistry, 1-3 Van Nostrand Reinhold Co.
- [5] <http://www.google.com>
- [6] <http://www.bartleby.com>>writing
- [7] <http://www.liveabout.com>
- [8] <http://en.m.wikipedia.org>
- [9] Chandrakant kokare, Pharmaceutical Microbiology, Experiment & Techniques; Career publication; 4<sup>th</sup> Edition; March 2014.
- [10] R. S. Gaud, Dr. G.D. Gupta; Practical Microbiology; Nirali Prakashan; 8th- Edition; March 2012.
- [11] [shodhganga.inflibnet.ac.in](http://shodhganga.inflibnet.ac.in)
- [12] <http://www.ukessays.com>
- [13] Official methods of analysis, Association of official analytical chemist publications, Washington.
- [14] Kaban G. Volatile compounds of traditional Turkish dry fermented sausage (sucuk). International Journal of Food Properties. 2010. Apr 30; 13(3): 525-34.
- [15] Szabo MR, Radu D, Gavrilas S, Chambre D, Iditoiu C. Antioxidant and antimicrobial properties of selected spice extracts. International Journal of Food Properties. 2010 Apr 30; 13(3): 535-45.
- [16] Davis DG. Instrumental methods of chemical analysis. (Ewing, Galen W.).
- [17] Analytical Chemistry an introduction, Skoog/West/Holler, 6<sup>th</sup> Edition.
- [18] Vogel Text Book of Practical Organic Chemistry- 5th Edition.
- [19] Pharmaceutical Analysis Vol.II. A. V. Kasture, /S. G. Wadhodkar,

K.R. Mahadik, H.N. More- Nirali  
Prakashan.

[20] Merck Index.

[21] Spectrometric Identification of  
Organic Compounds by  
Silverstein/Webster, 6th- Edition.