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RESEARCH ARTICLE

IDENTIFICATION OF BENZOIC ACID BY GC AND MASS SPECTROMETRY

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Abstract

Benzoic acid is an aromatic carboxylic acid naturally present in plant and animal tissues which can also be produced by microorganisms. Benzoic acid and a wide range of derivatives and related benzenoid compounds, such as salts, alkyl esters, parabens, benzyl alcohol, benzaldehyde and benzoyl peroxide, are commonly used as antibacterial and antifungal preservatives and as flavouring agents in food, cosmetic, hygiene, and pharmaceutical products. Benzoic acid (BA) is a commonly used antimicrobial preservative in food and beverages, especially in carbonated beverages, as it presents in its strongest antibacterial activity at pH 2.5–4.0. BA has inhibitory effects on the proliferation of bacteria and yeasts, a major cause of food spoilage. Although the addition of BA can extend the shelf life of drinks and prevent nutritional losses.Excessive intake of BA may cause diarrhea, abdominal pain, and other symptoms and even interfere with the intermediate metabolic processes of the body.

Introduction:-

Benzoic acid, a white, crystalline organic compound belonging to the family of carboxylic acids, widely used as a food preservative and in the manufacture of various cosmetics, dyes, plastics, and insect repellents. Benzoic acid exists in many plants; it makes up about 20 percent of gum benzoin, a vegetable resin. It was first prepared synthetically about 1860 from compounds derived from coal tar. It is commercially manufactured by the chemical reaction of toluene (a hydrocarbon obtained from petroleum) with oxygen at temperatures around 200° C (about 400° F) in the presence of cobalt and manganese salts as catalysts. Pure benzoic acid melts at 122° C (252° F) and is very slightly soluble in water. Among the derivatives of benzoic acid are sodium benzoate, a salt used as a food preservative; benzyl benzoate, an ester used as a miticide; and benzoyl peroxide, used in bleaching flour and in initiating chemical reactions for preparing certain plastics.

Polycyclic aromatic hydrocarbons consist of fused aromatic rings and do not contain heteroatoms or carry substituents. Two examples, pyrene and naphthalene, are shown in Figure 1. PAHs are pollutants widely found in our environment, and significant amounts of them can be found in crude oil and coal deposits. They are also produced as byproducts of fossil fuel burning and by incomplete combustion of carbon containing fuels. PAHs are relatively resistant to combustion, and their hydrophobicity hinders their degradation in liquid media. The larger PAHs are even less water soluble.

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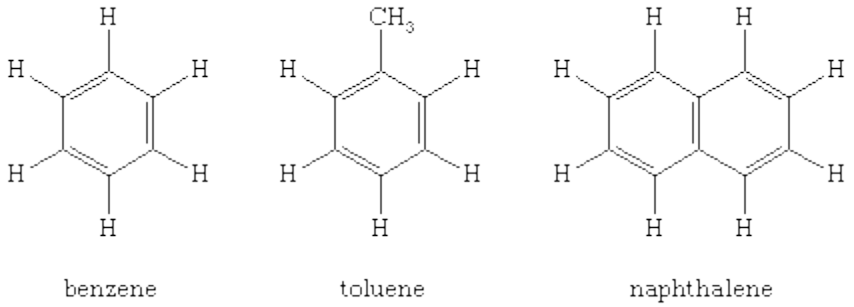


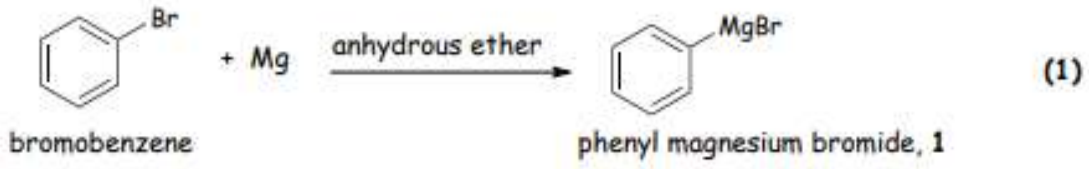
Figure 1:- Derivatives of benzoic acid.

Benzoic acid is an organic compound. Benzoic acid is present in our day-to-day life products ranging from food to cosmetics. It acts as a preservative for many products such as pickles, jams, lipsticks, face wash creams etc. It also acts as an essential precursor for many organic compound syntheses.The family of phenolic substances comprises a variety of compounds including flavonoids and phenolic carboxylic acids, and they have frequently been associated with the chemical defence of plants against microbes and as signal molecules in plant microbe interactions (Lynn and Chang, 1990; Mayer, 2004; Shaw et al., 2006). Many antimicrobial phenolic compounds have been identified, associated with allelopathic interactions. They are usually classified as either phytoalexins or phytoanticipins (Van Etten et al., 1994; Hammerschmidt, 1999). Although the effect of constitutive phenolics on fungal pathogens is well documented (Friend, 1985), it is not always clear whether the active agent is a specific phenolic compound or its metabolite (Scervino et al., 2006).

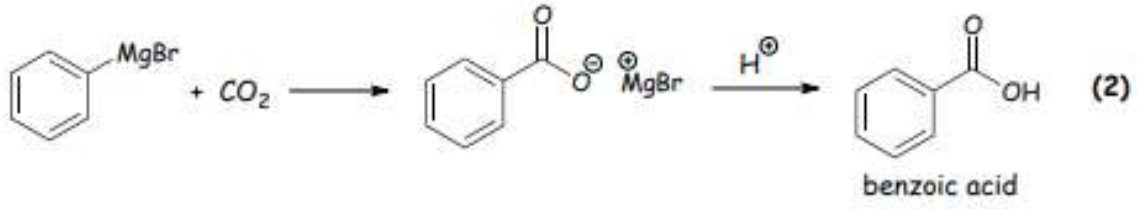
Method And Material:-

Synthetic Procedure for Preparationof benzoic acid

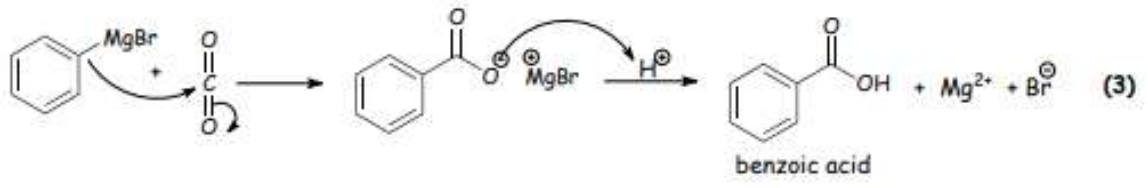
The experiment begins with the preparation of a Grignard reagent, phenylmagnesium bromide, from bromobenzene and magnesium metal using diethyl ether ("ether") as the solvent under anhydrous conditions. The use of an ether, such as diethyl ether, as a solvent for the preparation of Grignard reagents is of key importance because the lone pairs of electrons on oxygen help stabilize the partial positive charge on magnesium in the Grignard reagent and thus facilitate its formation. Grignard reagents do not form in most other "inert" solvents. In general, ethers like diethyl ether or tetrahydrofuran (THF) work well as solvents in many nucleophilic addition reactions because the ether functional group is unreactive with most reactants.



Phenyl magnesium bromide will then be allowed to react with CO2, followed by hydrolysis, to give benzoic acid [equation (2)].



The synthetic use of the Grignard reagent, phenylmagnesium bromide, in this experiment is its addition to carbon dioxide to produce, after hydrolysis, benzoic acid [equation (2)]. The mechanism for this reaction is provided in equation (3). A convenient source of CO_2 is Dry-Ice (solid CO_2) which will be used in this experiment.



Gas Chromatography Separation

SeparationbyGCoccurswithin thecolumn.The sample containing multiple compounds is injected into the column together with the mobile phase. (In GC, the mobile phase is a gas referred to as the carrier gas. He is frequently used.) Both the sample and the mobile phase travel through the column, but the rate of progression within the column differs depending on the compound. Accordingly, differences arise in the times at which the respective compounds arrive at the column outlet. As a result, a separation between each compound occurs. The row of peaks drawn when the electrical signals output from the GC detector are plotted on the vertical axis and the elapsed time after sample injection is plotted on the horizontal axis is calledachromatogram.The components passing through the column are transported by the mobile phase (gas phase) while being partitioned from and adsorbed into the stationary phase (liquid phase and solid phase).

Mass Spectroscopy-

Mass spectrometry (MS) is an analytical technique that separates ionized particles such as atoms, molecules, and clusters by using differences in the ratios of their charges to their respective masses (mass/charge; m/z), and can be used to determine the molecular weight of the particles. MS instruments consist of the following modules: an ion source, which splits the sample molecules into ions; a mass analyzer, which sorts the ions according to their masses by applying electromagnetic fields; a detector, which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present; and a computer, which regulates the mass analyzer and manages the data derived from thedetector. In these modules, the prevailing techniques for the ion source and mass analyzer have several variations, and selecting their appropriate combination is particularly important for IMS.

Antibacterial Activity-

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this review, we focused on the use of antimicrobial testing methods for the **in vitro** investigation of extracts and pure drugs as potential antimicrobial agents. A variety of laboratory methods can be used to evaluate or screen the **in vitro** antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism.

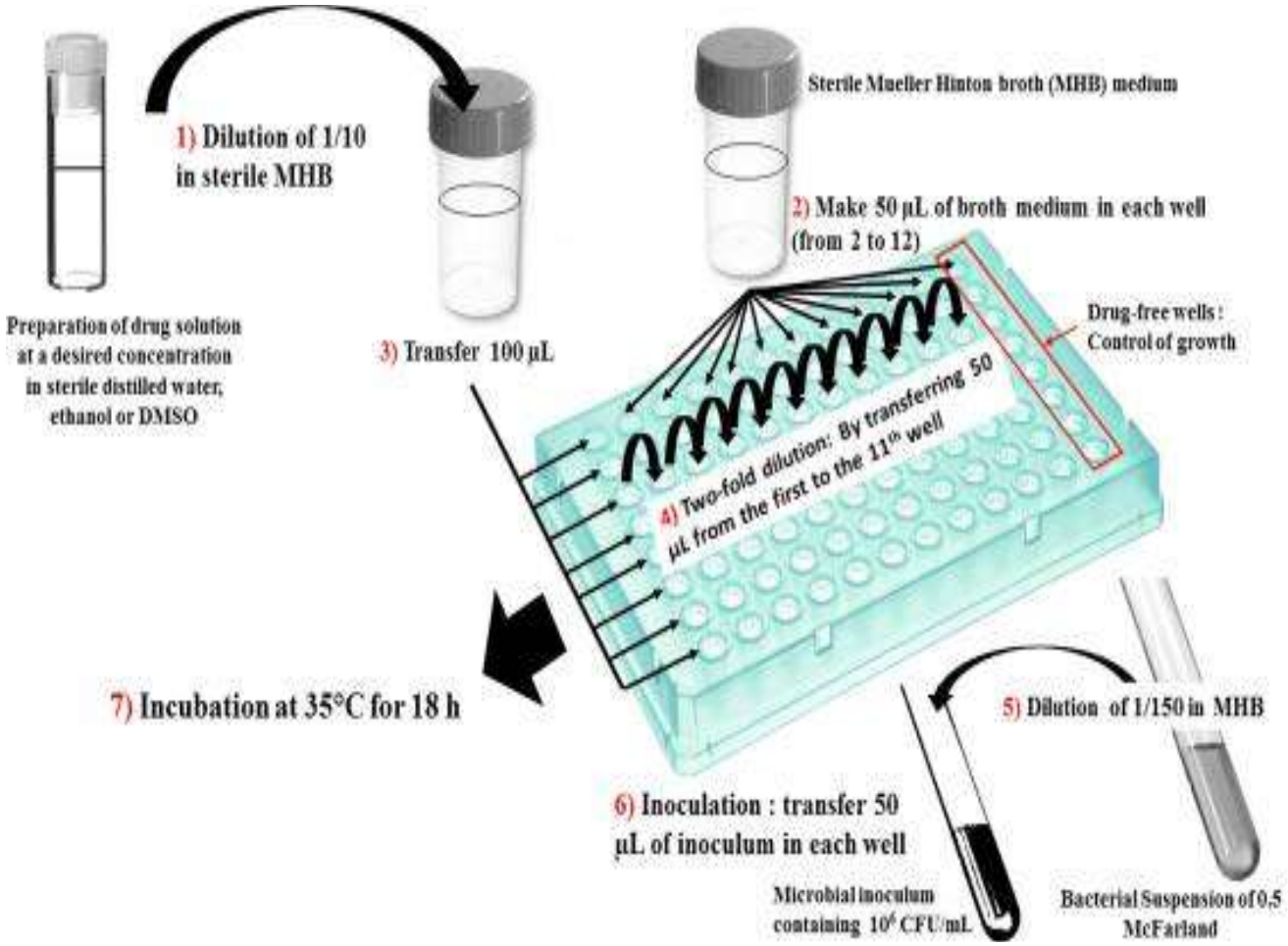


Figure2:- Serial dilution method in sterile MHB.

Result And Discussion:-

Optional GC/MS analysis of by-products in the organic layer-

The mechanism for the formation of a Grignard reagent, and of some of the byproducts, is shown below. Notice that the reaction is initiated at the surface of the metal by an electron transfer to the aryl halide. This reduction process results in the formation of a bromide anion and a phenyl radical, a highly energetic, reactive intermediate. This molecule results when the bromide anion is removed from the bromobenzene radical anion. The resulting unpaired electron remains in the vacant sp2 hybrid orbital. This free-radical is very similar to the familiar alkyl radical such as methyl, with an unpaired electron in the sp3 orbital. It is the phenyl radical that reacts with Mg+1 to generate phenyl magnesium cation that then traps a bromide anion to form the Grignard reagent.

Phenyl radical may undergo side reactions typical of organic radicals, such as reaction with another phenyl radical. This occurs when the localized concentration ofradicals is too high. The resulting possible by-products will be identified by the GC/MS analysis of the ether. Recall that the Pre-Lab exercise asked that you make some suggestions as to the nature of these by-products based on your understanding of radical reactions. Now make use of GC/MS data to validate or refute your previous hypothesis. The understanding of these side reactions will help in planning of experimental synthetic procedure. Obviously, minimizing the former will increase the yield of the desired Grignard reagent and ultimately the desired product.

Table1:- Precise data for benzoic acid residue on the basis of days.

Concentration of Benzoic acid (µg/g)	Sample No	Day 1		Day 2		Day 3		Intra-assay	
		Benzoic acid conc. obtained (µg/g) n=5	% RSD	Benzoic acid conc. obtained (µg/g) n=5	% RSD	Benzoic acid conc. obtained (µg/g) n=5	% RSD	Benzoic acid conc. obtained (µg/g) n=5	% RSD
0.1	1	0.100	3.72	0.104	3.55	0.103	3.65	0.102	3.62
	2	0.101	3.56	0.102	3.48	0.099	3.38	0.101	3.55
	3	0.099	3.82	0.100	3.21	0.101	3.46	0.099	3.28
0.5	1	0.503	2.82	0.501	2.76	0.504	2.92	0.501	2.71
	2	0.500	2.75	0.502	2.86	0.502	2.85	0.500	2.82
	3	0.499	2.12	0.501	2.81	0.500	2.69	0.499	2.52
5.0	1	5.06	1.25	5.05	1.42	5.09	1.21	5.05	1.35
	2	5.08	1.31	5.04	1.34	5.05	1.60	5.07	1.27
	3	5.00	1.02	4.99	1.38	5.01	1.52	5.00	1.20

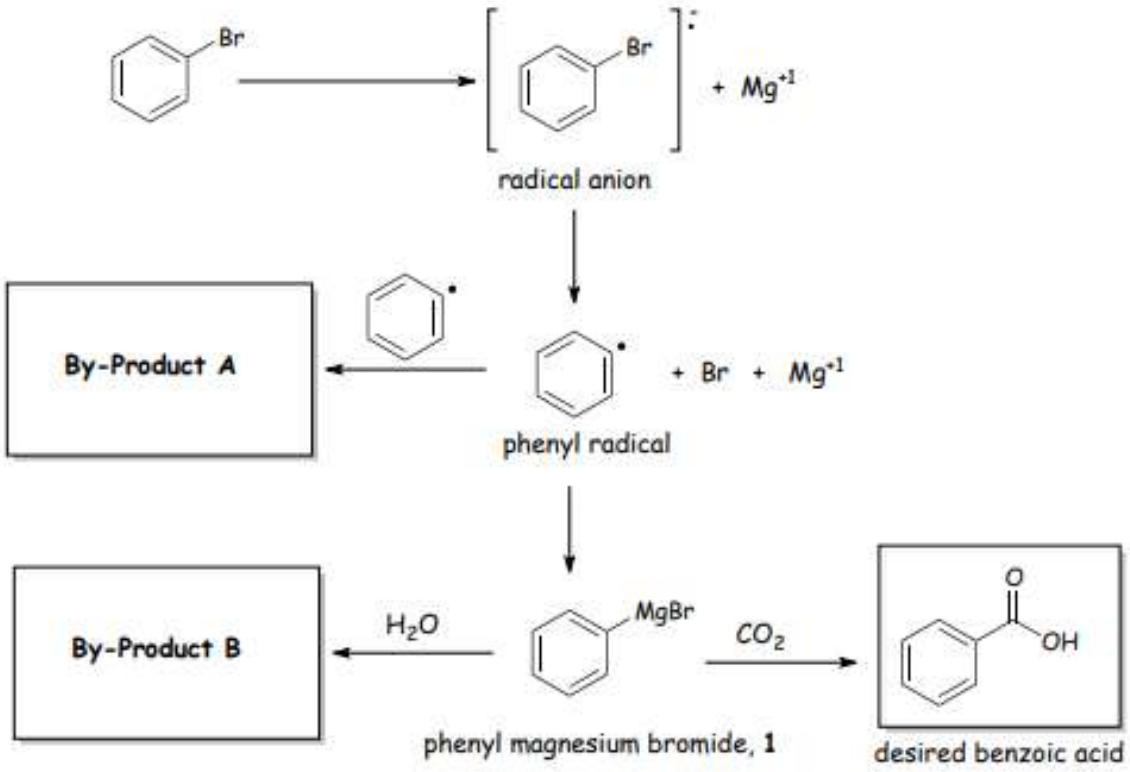


Figure3:- Shows formation of benzoic acid from phenyl Grignard reagent.

Table2:- Benzoic acid obtained at different days.

Spiking level µg/g	Sample No 1	Day1		Day 2		Day 3	
		Amount calculated µg/g	% Recovery	Amount calculated µg/g	% Recovery	Amount calculated µg/g	% Recovery
1.0	1	0.971	97.1	1.031	103.1	0.999	99.9
	2	0.982	98.2	1.024	102.4	1.012	101.2
	3	0.991	99.1	0.998	99.8	1.041	104.1
2.0	1	2.06	103.0	1.98	99.0	2.02	101.0
	2	2.04	102.0	1.99	99.5	2.01	100.5
	3	2.01	100.5	2.00	100.0	2.02	101.0

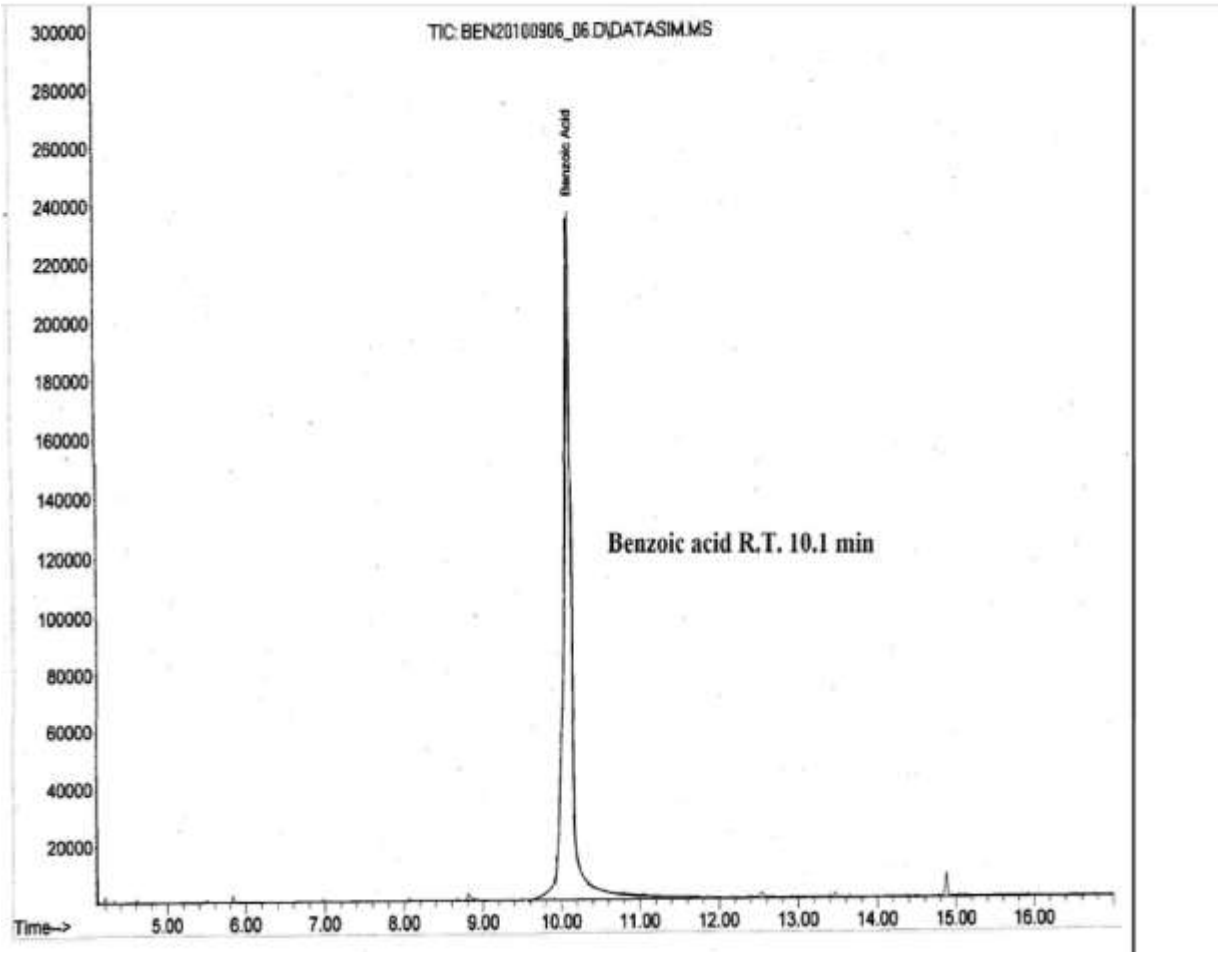


Figure4:- Chromatogram obtained from synthetic benzoic acid.

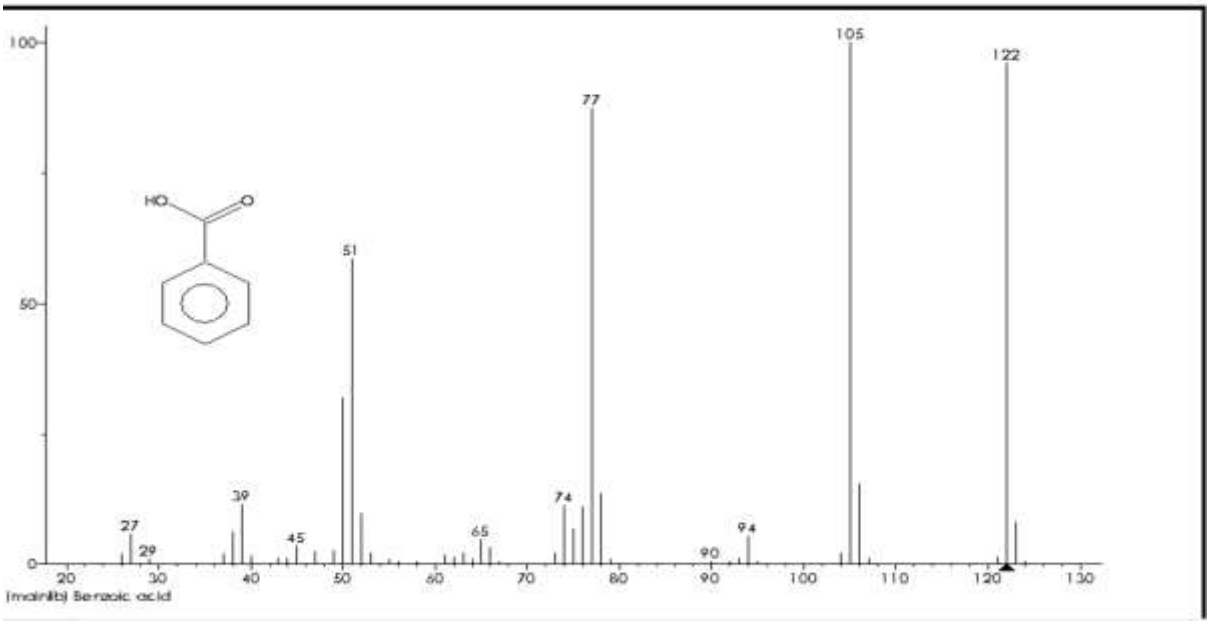


Figure 5:- Mass spectra of benzoic acid from synthetic benzoic acid.

The recovery of benzoic acid in spiked samples was calculated to study theeffect of matrix on the determination of benzoic acid. The recovery studies were carried out attwo differentconcentrations. For this two different portions of pre-analyzed three different samples were spiked with 1.0 mg/g and 2.0 mg/g respectively in six replicates on threedifferent days and then extracted and determined by the same method as mentioned earlier. Therecoveries of benzoic acid from synthestic samples were evaluated on the basis of thecomparison of the theoretical concentration level of the spiked solutions with the observed.Robustness of the method was determined by analyzing the same set of spikedsamples (i.e. samples spiked at concentration levels of 0.1 mg/g, 0.2 mg/g and, 0.5 mg/g) underdifferent parameters; such as same column chemistry from different manufacturers, differentanalysts, and different injection volumes. The method was found to be robust even with smallchanges in analytical conditions: change in flow rate (± 0.1 ml/min), a change in injectortemperature (+ 2°C), use of same column from different manufacturers (HP5, DB5, CP-sil 8CB). Under all of these conditions, the analytical values of the spiked samples were not affectedand it was in accordance with the actual values.

Minimum Inhibitory Concentration-

The response (MIC) is recorded in terms of zone of inhibition and inhibitory concentration. It is shown in the table-3 thatMinimum zone of inhibition is around 23mm in *E.coli* and 19 mm in *Bacillus* at diffferent dilution concentration of plant extract in methanol extract while 10mm in *E.coli* and 7 mm in *Bacillus subtilis* in ethanol extract were recorded.

Table3:-Zone of inhibition obtained against two bacteria *B.subtilis* and *E.coli*.

S.No.	**Solvent**	**Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation*Bacillus subtilis*and *E.coli***		
		0.75mg	**0.5mg**	**0.25mg**
1	Methanol	**19** + 0.6	**17**+ 0.6	**14** + 0.6
2	Ethanol	**7** + 0.2	**8** + 0.4	**5** + 0.2

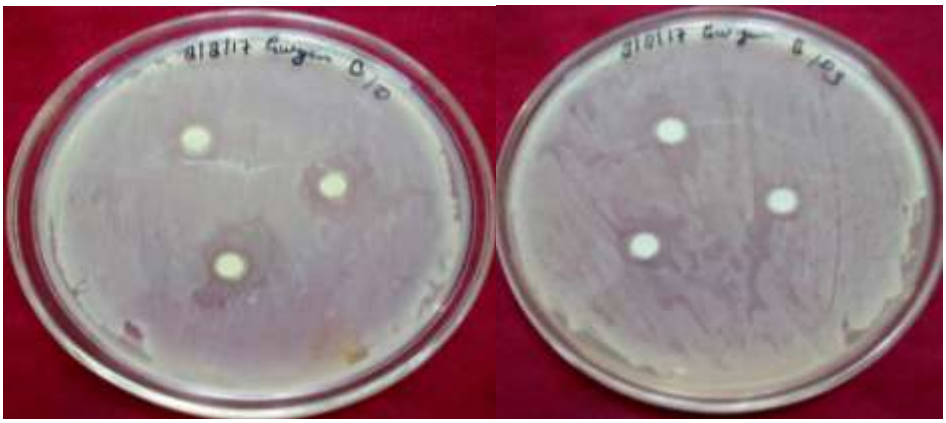


Figure6:- MIC zone obtained from benzoic acid concentration available in sample.

Conclusion:-

Benzoic acid is used as a preservative in plant samples but it should be present at ppm level becauseat high concentration it affects to the human health. A validated gas chromatography-Massspectrometer and GC method has been developed for the determination of benzoic acid atppm level. The work described in this paper has shown that the analytical method developed isprecise, accurate, sensitive and robust for the determination of benzoic acid. The method isspecific to the analysis of synthetic benzoic acid without any matrix interference.Phenolic compounds have long been considered potent strong antioxidants which act in vitro. The relationship between their structure and antioxidant activity and dosage is very important. In some studies, they have been shown to be more effective antioxidants than vitamin E, vitamin C and carotenoids. A balanced diet rich in fruits and vegetables with high content of natural antioxidants available in benzoic acid has been shown to reduce the risk of chronic diseases such as malignancy, diabetes mellitus, Alzheimer's disease, cardiovascular diseases.

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