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Research Article **Chemistry**

Synthesis of Benzocain Compound Using Microwave Technology and Evaluation of its Antibacterial Activity

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ABSTRACT

Aim of the study to synthesis of benzocain compound using microwave technology and evaluation of its antibacterial activity. Benzocain is the prime example of anesthetic agent. According to reported procedure, it is synthesized by condensation of amino benzoic acid and HCl using ethanol as solvent which itself acts as CNS depressant. Synthesized Benzocain further confirmed in UV spectrum. Synthesized Benzocain further evidenced by FTIR. Fluorescence behavior of Synthesized Benzocain was examined. Antibacterial activity of Benzocain confirmed against *Escherichia coli* and *Staphylococcus aureus*. Over all, Benzocain is synthesized by application of principle of green chemistry as well as having safety by the use of ethanol. There is reduction in time and ultimately cost in the use of microwave procedure of synthesis of Benzocain and also a potential antibacterial agent.

Keywords: Benzocain, Antibacterial activity, Amino benzoic acid and HCl

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INTRODUCTION

The organic synthesis is one of the major role of research in chemistry, from plastics to medication it participates in the improvements of everyone life. Over the past few decades, many significant advances in practical aspects of organic chemistry have included novel synthetic strategies and methods as well as advent of a vast array of analytical techniques. The first methods are as old as chemistry itself, their use by synthetic chemist has gained importance only in the past decade. With easy availability of ultrasound and microwave sources, their use in chemistry has gained momentum recently (Desai., 2005).

Microwave have been used to speed up chemical reactions in the

Laboratories (Mingos., 1994) which led scientists to investigate the mechanism of microwave dielectric heating and to identify the advantages of the technique for chemical synthesis.(Hoz de la., 2005) During recent years, microwaves have been extensively used for carrying out chemical reactions and have become a useful non-conventional energy source for performing organic synthesis (Gedye *et al*., 1998). This is supported by a great number of publications in recent years, particularly in 2003, related to the application of microwaves as a consequence of a great availability of dedicated and reliable microwave instrumentation.(Mingos., 1997; Nuchter., 2000; Nuchter., 1996).

Benzocaine is a local anesthetic commonly used as a topical pain reliever or in cough drops. It is the active ingredient in many over-the-counter anesthetic ointments such as products for oral ulcers. It is also combined with antipyrine to form A/B otic drops to relieve ear pain and remove earwax (Demare *et al*., 2012) .Benzocaine is the ethyl ester of p-aminobenzoic acid (PABA). It can be prepared from PABA and ethanol by Fischer esterification or via the reduction of ethyl p-nitrobenzoate (Dans-Lax *et al*., 1982).

Benzocaine is indicated to treat a variety of pain-related conditions. It may be used for Local anesthesia of oral and pharyngeal mucous membranes (sore throat, cold sores, mouth ulcers, toothache, sore gums, denture irritation, Otic Pain (earache) and Surgical or procedural local anesthesia.

Microwave-assisted organic synthesis is an enabling technology for accelerating drug discovery and development processes. Microwave organic synthesis opens up new opportunities to the synthetic chemist in the form of new reaction that are not possible by conventional heating and serve a flexible platform for chemical reaction. In the present study to synthesis of benzocain compound using microwave technology and evaluation of its antibacterial activity.

MATERIALS AND METHODS

Synthesis of Benzocain compounds using Microwave Technology

Benzocain synthesized broom temperatue and the producty the method of Shrinivas (Shrinivas *et al*., 2013). A mixture of *p* amino benzoic acid (3gm), ethanol(20ml) and conc. Hydrochloric acid (HCl) taken in a 100ml conical flask. After coverting with funnel, the mixture was irradiated with microwaves at 60% (540W) intensity for 60 seconds. A beaker containing water was placed in the oven next to reaction vellel to serve as a heating sink. Then reaction product was cooled to room temperature the product was poured onto cold water, neutralized with sodium carbonate and the separated product was filtered, washed with water, dried and recrystallized from ethanol. Synthesized Benzocain further used for UV Vis., FTIR Spectrum and antibacterial activity.

UV-Visible analysis

The Benzocain was examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The Benzocain were scanned in the wavelength ranging from 330-920 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 50 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

Determination of Fluorescence behavior of plant powder (Rao *et al.,* 2011)

Fluorescence analysis of Benzocain powder has been carried out in daylight and under U.V light. Florescence analysis of powder of Benzocain was carried out by the treatment of different chemical reagents such as methanol, H_2SO_4, HCl, HNO_3, NaOH, acetone, hexane, chloroform and distilled water. The powders were observed in normal daylight and under short (245nm) and long U.V. light (365 nm).

Determination of antimicrobial activity

Antibiogram was done by disc diffusion method (NCCLS., 1993; Awoyinka *et al.*, 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing *Escherichia coli* and *Staphylococcus aureus* spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the Benzocain (50µl, 100 µl and 150 µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37°C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the Benzocain were measured using a millimeter scale.

RESULTS AND DISCCUSSION

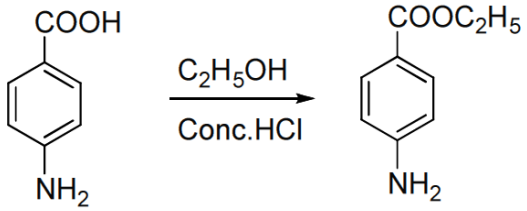
Traditionally, organic synthesis is carried out by conductive heating with an external heat source. This is a comparatively slow and inefficient method for transferring energy into the system, since it depends on the thermal conductivity of the various materials that must be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture (Kappe., 2004).

Microwave-enhanced chemistry is based on the efficient heating of materials by "microwave dielectric heating" effects. This phenomenon is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat1 . Microwaves are defined as electromagnetic waves with vacuum wavelength ranging between 0.1to 100cm or, equivalently, with frequencies between 0.3 to 300GHz. Although the first reported by group of (Gyedye and Gigure Majetih., 1986), the use of microwaves in organic synthesis was initially hampered by a lack of understanding of the basic principal of MW heating and the inability to obtain reproducible results with domestic microwave oven (Jain and Singla., 2011). With microwave heating, the energy can be applied directly to the sample rather than conductively, via the vessel. Heating can be started or stopped instantly, or the power level can be adjusted to match the required.

The interest in the microwave assisted organic synthesis has been growing during the recent years. Drug companies are exploiting microwave in the area of organic/pharmaceutical synthesis for drug screening and discovery. Microwave heating is also called as green chemistry and the development of cleaner technologies is a major emphasis in green chemistry. Among the several aspects of green chemistry, using efficient and less hazardous energy sources such as microwave energy is recommended (Barchin *et al*., 2012). In the presemt study to synthesis the Benzocain.

Benzocain has been synthesized by condensation of amino benzoic acid and HCl in the presence of a solvent. Synthesis of Benzocain is carried out by reacting amino benzoic acid and HCl in the presence of ethanol. The reaction takes place in the presence of other catalytic base such as sodium hydroxide.

Mechanism of Benzocain formation



p-Amino benzoic acid Benzocain

Microwave radiation, an electromagnetic radiation, is widely use as a source of heating in organic synthesis. The basic mechanisms observed in microwave assisted synthesis are dipolar polarization and conduction. Microwave assisted organic synthesis (MAOS) has emerged as a new "lead" in organic synthesis.

Fig 2 Synthesized Benzocain

Ultraviolet/visible (UV/VIS) spectroscopy

The relative percentage of scatter or absorption from the measured extinction spectrum depends on the size, shape, and composition and aggregation state of sample. Sample may absorb light, scatter light, or both. As a general rule, smaller particles will have a higher percentage of their extinction due to absorption.

Scattering from a sample is typically very sensitive to the aggregation state of the sample, with the scattering contribution increasing as the particles

aggregate to a greater extent. For example, the optical properties of silver nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighbouring particles. When this occurs, the surface plasmon resonance shifts to lower energies, causing the absorption and scattering peaks to red-shift to longer wavelengths. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the organic compounds. The peaks 420 indicate the aromatic ring of the synthesized compound (Fig 3).

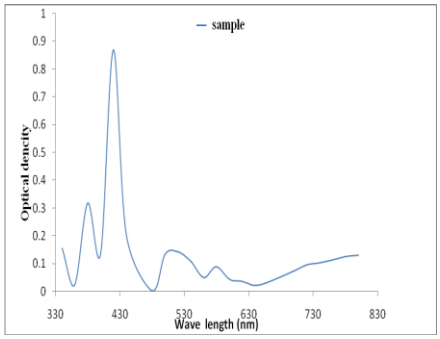


Fig 3: UV Vis, spectrum of Benzocain

IR Spectrum

IR spectrum can observe some characteristic absorption bands of various important groups in the structure of Benzocain. The following IR bands, observed in the FT IR spectra of Benzocain and its β-CD complex, see **Fig. 4,** were assigned: O-H stretching band (between 3300 and 3900 cm-1, broad band), N-H stretching band (3300-3500 cm-1), aliphatic C-H stretching bands (2850-2960 cm-1), aromatic C-H stretching bands (2900-3100cm-1).

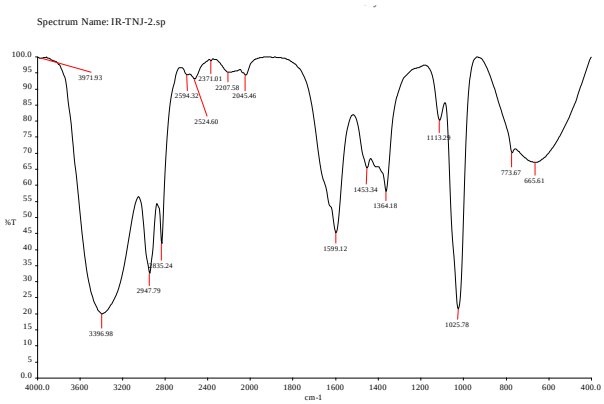


Fig 4: IR spectrum of Benzocain

Fluorescence behavior

Fluorescence behavior of Benzocain was investigated by addition of acid or alkali. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many products, which do not visibly fluoresce in daylight. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents. Hence, some drugs are often assessed qualitatively in this way and it is an important parameter of potential use as an imaging agent. Table 1 represents Fluorescence behavior of Benzocain powder

Table 1: Fluorescence behavior of Benzocain

S.NO	Test	Visible	Short Wavelength	Long Wavelength
1	Drug powder	White	White	Brown
2	Drug Powder + Distilled Water	Light White	Light White	Brown
3	Drug Powder + Hexane	White	White	Brown
4	Drug Powder + $CHCl_3$	Light White	Light White	Dark Brown
5	Drug Powder + CH_3OH	Light White	Light White	Brown
6	Drug Powder + Acetone	White	White	Dark Brown
7	Drug Powder + NAOH in H_2O	White	White	Dark Brown
8	Drug Powder + 1N HCl	Light Yellow	Light Yellow	Dark Brown
9	Drug Powder + H_2SO_4 in H_2O	White	White	Light Brown
10	Drug Powder + HNO_3 in H_2O	Light Yellow	Light Yellow	Black

Antibacterial activity of Benzocain

Toxicity studies on pathogen opens a door for organic chemistry applications in medicine. *Staphylococcus aureus* is a Gram-positive extracellular bacterium that is the most common cause of skin and soft tissue infections,such as cellulitis, impetigo, and folliculitis (Todar., 2007). *Escherichia coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In some cases, virulent strains are also responsible for haemolyticuremic syndrome, peritonitis, mastitis, septicaemia and pneumonia (McCaig 2006).

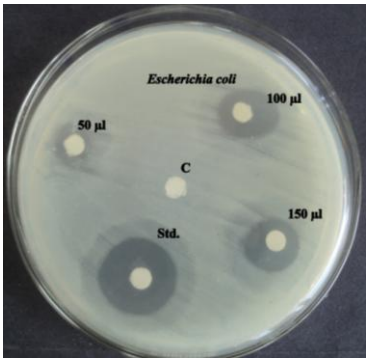
Synthesis of Benzocain is a microwave method and the use of amino benzoic acid and HCl has a new awareness for the control of disease, besides being safe. The synthesized Benzocain was found to be highly toxic against different pathogenic bacteria of selected species. The Benzocain shows highest antibacterial activity was observed against *Escherichia coli* than *Staphylococcus aureus*. The inhibitory activities in culture media of the Benzocain reported in table 2 and fig 6 were comparable with standard antimicrobiotic viz. chloromphenical.

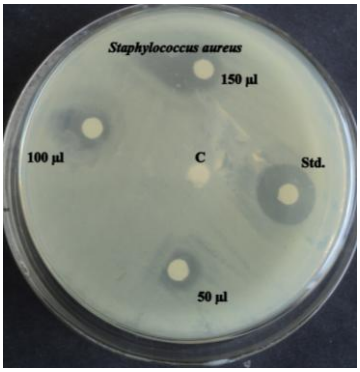
Table 2Antibacterial activity of Benzocain

Microorganisms	50 µl	100 µl	150 µl	Standard (Chloromphenical for bacteria)	Control (solvent)
Escherichia coli (mm)	1.05±0.07	2.13±0.14	3.98±0.27	6.54±0.45	0
Staphylococcus aureus (mm)	1.03±0.07	2.17±0.15	3.65±0.25	6.73±0.47	0

Fig 6: Antibacterial activity of Benzocain

Escherichia coli ***Staphylococcus aureus***





CONCLUSION

Microwave assisted organic synthesis has revolutionized organic synthesis.. As a result, this technique has rapidly gained acceptance as a valuable tool for accelerating drug discovery and development processes. Benzocain is the prime example of anesthetic agent. According to reported procedure, it is synthesized by condensation of amino benzoic acid and HCl using ethanol as solvent which itself acts as CNS depressant. The following conclusion obtained from the study are Benzocain synthesized by condensation of amino benzoic acid and HCl. Synthesized Benzocain further confirmed in Uv spectrum. Synthesized Benzocain further evidenced by FTIR. Fluorescence behavior of Synthesized Benzocain was examined. Antibacterial activity of Benzocain confirmed against *Escherichia coli* and *Staphylococcus aureus*. Over all, Benzocain is synthesized by application of principle of green chemistry as well as having safety by the use of ethanol. There is reduction in time and ultimately cost

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