World Journal of Science and Research. 1(2): 26-30

ISSN: 2455 2208

Available online at http://www.harmanpublications.com

World Journal of Science and Research



Research Article

Chemistry

# SYNTHESIS OF PHENYTOIN COMPOUND USING MICROWAVE TECHNOLOGY AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

P. Sangeetha[1], T. Siva[1], R. Balaji[1] and K. Tharini[2]

[1]Department of Chemistry, Rajah Serfoji Govt. College, Thanjavur, Tamil Nadu, India
[2]Department of Chemistry, Government Arts College, Thiruverumbur, Tamil Nadu, India
.

**ABSTRACT**

The aim of the study to synthesis phenytoin compound using microwave technology and evaluation of its antibacterial activity. Phenytoin is an antiepileptic drug that can be used in the treatment of epilepsy. Phenytoin synthesized by condensation of benzil and urea in presence of base. Synthesized Phenytoin further confirmed in UV spectrum. Synthesized Phenytoin further confirmed in FTIR. Fluorescence behavior of Synthesized Phenytoin was examined. Antibacterial activity of Phenytoin confirmed against Escherichia coli and Staphylococcus aureus. Over all, Phenytoin 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 phenytoin and also a potential antibacterial agent.

***Citation***: P. Sangeetha, T. Siva, R. Balaji and K. Tharini. (2016) Synthesis of phenytoin compound using microwave technology and evaluation of its antibacterial activity. World Journal of Science and Research. 1 (2): 26-30.

***Article Info:***

Received on 10 April 2016
Accepted on 25 May 2016
Online May 2016

***Keywords:***

Phenytoin, Organic synthesis, Microwave, Antibacterial activity, Benzil and Urea.

***Corresponding author***
P. Sangeetha, Department of Chemistry, Rajah Serfoji Govt. College, Thanjavur, Tamil Nadu, India.

## INTRODUCTION

Phenytoin is an antiepileptic drug that can be used in the treatment of epilepsy. The main site of action appears to be the motor cortex where the spread of seizure activity is inhibited (Kruer *et al*., 2013). By promoting the flow of sodium in neurons, phenytoin tends to settle against hyperexcitability threshold caused by excessive stimulation, or changes in the environment capable of reducing the sodium in the membrane. Phenytoin reduces the maximal activity of seizures (Rogawski and Löscher, 2004). Phenytoin also is used to control arrhythmias (irregular heartbeat) to treat migraines, headaches and pain of the facial nerve (Balaji., 2004). According to the current level of knowledge and therapeutic means, epilepsy medication orders is missing or at least significantly reduce seizures and adequate quality of life, in terms of minimal side effects of antiepileptic drugs (Balaji, 2004).

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., 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 *et al*., 1997; Nuchter *et al*., 2000; Nuchter *et al*., 2001). 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. The aim of the study to synthesis phenytoin compound using microwave technology and evaluation of its antibacterial activity.

## MATERIALS AND METHODS

**Plant materials:**

**Synthesis of Phenytoin compounds using Microwave Technology**

Phenytoin synthesized by the method of Shrinivas *et al*., (2013). A mixture of benzyl (2.65 g), Urea (15gm), 30% NaOH (7.5ml) and ethanol(37.5ml) was taken in a 100ml conical flask and mixed. A funnel was placed over the flask to avoid excessive evaporation. The reaction mixture was irradiated with microvaves 60% (540W) intensity for 150 seconds. A beaker containing water was placed in the oven next to reaction product was cooled to room temperature. Reaction mixture was poured onto 100ml of distilled water and mixed thoroughly. To the resulting solution conc. HCl was added till the solution becomes acidic and precipitation of Phenytoin occurred. It was cooled for complete precipitation. The separated product was filtered, dried and recrystallized from alcohol. Synthesized Phenytoin further used for UV Vis. Spectrum and antibacterial activity.

**UV-Visible analysis**

The Phenytoin was examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The Phenytoin 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 Phenytoin powder has been carried out in daylight and under U.V light. Florescence analysis of powder of Phenytoin was carried out by the treatment of different chemical reagents such as methanol, H2SO4, HCl, HNO3, 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 Phenytoin (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 Phenytoin were measured using a millimeter scale.

## RESULTS AND DISCUSSION

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).

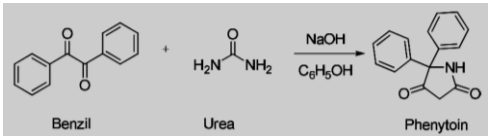
Microwave-enhanced chemistry is based on the efficient heating of materials by “microwave dielectric heating” effects. This phenomenon is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat1 . Microwaves are defined as electromagnetic 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., 1886), 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*.,2002). In the presemt study to synthesis the phenytoin.

Phenytoin has been synthesized by condensation of benzyl urea in the presence of a solvent. Synthesis of 5, 5-diphenylhydantoin (phenytoin) is carried out by reacting benzyl, and urea in the presence of sodium hydroxide. The reaction takes place in the presence of other catalytic base such as sodium hydroxide.

**Mechanism of Phenytoin formation**




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.

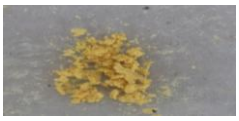


**Fig 2 Synthesized Phenytoin**

**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 380 and 700 indicate the aromatic ring of the synthesized compound.

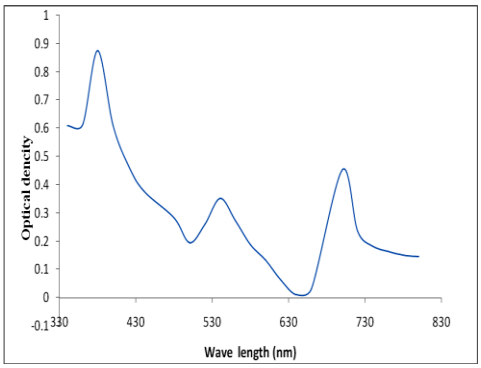



**Fig 3:UV Vis, spectrum of Phenytoin**

**FTIR Spectrum**

IR spectrum can observe some characteristic absorption bands of various important groups in the structure of phenytoin. Thus, peaks at 3385 cm-1 (high intensity) and at 3352 cm-1 corresponding to υN-H stretching vibration, and the vibration at 3074 cm-1 stretch υ=CH arom. There are high intensity peaks at 1650 which are attributable to the stretching vibration of C=O grouping, υC=O (ketone). Peaks appear at 1494 cm-1 (w) attributable to the stretching vibrations υC=C aromatic 1454 and 1412 cm-1, characteristic absorption bands δN-H bending vibrations. The spectrum peaks appear at 1111 cm-1, 1025 cm-1 (w) which are characteristic υC-C stretching vibration. In the 700 cm-1 there are characteristic absorption bands δC-H bending vibrations (characteristic flavors) and monosubstituted phenyl ring vibrations.

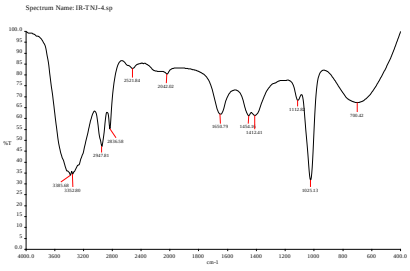


**Fig 4: FTIR spectrum of Phenytoin**

**Fluorescence behavior**

Fluorescence behavior of Phenytoin 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 Phenytoin powder

**Table 1:Fluorescence behavior of Phenytoin**

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

**Antibacterial activity of Phenytoin**

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).

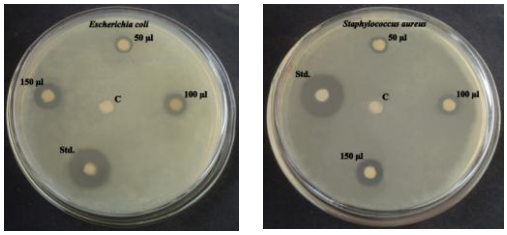
Synthesis of Phenytoin is a microwave method and the use of benzyl and urea has a new awareness for the control of disease, besides being safe. The synthesized Phenytoin was found to be highly toxic against different pathogenic bacteria of selected species. The Phenytoin shows highest antibacterial activity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

**Table 2Antibacterial activity of Phenytoin**

| Microorganisms | 50 µl | 100 µl | 150 µl | Standard (Chloromphenical for bacteria) | Control (solvent) |
|---|---|---|---|---|---|
| *Escherichia coli* (mm) | 1.52± 0.10 | 2.98± 0.20 | 4.17± 0.29 | 6.83±0.47 | 0 |
| *Staphylococcus aureus* (mm) | 1.23± 0.18 | 2.76± 0.19 | 4.11± 0.28 | 6.92±0.48 | 0 |

**Fig 6:Antibacterial activity of Phenytoin**

***Escherichia coli*** ***Staphylococcus aureus***




## CONCLUSION

Microwave assisted organic synthesis has revolutionized organic synthesis. Small molecules can be built in a fraction of the time required by classical thermal methods. The following conclusions obtained from the study are Phenytoin synthesized by condensation of benzil and urea in presence of base. Synthesized Phenytoin further confirmed in UV spectrum. Synthesized Phenytoin further confirmed in FTIR. Fluorescence behavior of Synthesized Phenytoin was examined. Antibacterial activity of Phenytoin confirmed against Escherichia coli and Staphylococcus aureus. Over all, Phenytoin

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 phenytoin and also a potential antibacterial agent.

## Acknowledgement

The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www.harmanresearchcentre.com), Thanjavur, Tamil Nadu for providing necessary facility to complete this work.

## REFERENCES

Awoyinka Awoyinka O, Balogun IO and Ogunnowo AA. (2007) Phytochemical screening and invitro–Vanderhoff JW; U. S. 3; 1969; 432- 413;

Balaji S. (2004)"Medical therapy for sudden death", Pediatr Clin North Am., 51(5): 1379-87.

injury: A shift from phenytoin to levetiracetam", Journal of Critical Care, 28: 883.

McCaig. (2006) J. Chem. Res, 420–425.

Mingos DMP, Whittaker AG, Van RV, Malik C and Hubbard D.(1997) John Wiley and Spektrum Akademischer Verlag Co- Publication, New York and Heidelberg, 479.

NCCLS. (1993) National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. PA: NCCLS Publications 2-5.

Nuchter M, Ondruschka B, Jungnickel A, Muller U, Phys J. (2000) Org. Chem, 13; 586.

Nuchter M, Ondrushka B and Lautenschlager W. (2001)Synth. Commun, 37; 1277

Barchin BM, Cuadro AM and Builla JA.(2002) Synlett, 2:1437-2096.

DMP Mingos. (1994) Chem. Ind. 596-599.

Gedye RN and Wei JB. (1998) Can. J. Chem, 76: 525–527. (b) RS Varma; Green 1999; 1; 43–55.

Gyedye and Gigure Majetih. (1986) Indian J. Chem.; Sec. B.; 45B; 2305–2307.

Hoz de la A, Dfaz A, and Moreno A. (2005) Chem. Soc. Re; 34: 164-178.

Jain AK and Singla RK.(2011) Webmed Centra, 1-15.

Kappe C O. (2004) Angew Chem Int, 43: 6250 – 6284.

Kappe CO. (2004) Angew Chem Int Edn; 43: 6256.

Kruer RN, Harris LH, Goodwin H, Kornbluth J, Thomas KP, Slater LA and Haut ER. (2013)"Changing trends in the use of seizure prophylaxis after traumatic brain

Rao KNV, Sunitha Ch, David Banjii, Sandhya S and Saikumar P. (2011) Pharmacognostic Studies on Rumex vesicarius. Asian Journal of Plant Science and Research, 1 (1):102-115.

Rogawski MA and Löscher W. (2004) "The neurobiology of antiepileptic drugs", Nat Rev Neurosci., 5(7) :553-64.

Shrinivas JD, Manoj K and More Uttam A. (2013) Synthesis of some medicinal/organic compounds using microwave technology. Universal Journal of Pharmacy, 02(03): 139-143.

Todar. (2007) Chimica Oggi, 25: 20–26.

**Source of support: Nil;**
**Conflict of interest: None declared**