Research Article

Microwave Assisted Synthesis, Characterization and Evaluation of Antimicrobial Activity of 1, 3, 4-thiadiazole Derivative of Guar gum

Prof. Sangeeta Loonker, Akanksha Maheshwari*
Department of Chemistry, Jai Narain Vyas University, Jodhpur (Rajasthan), India.
***Corresponding author's E-mail:** maheshwari.akanksha90@gmail.com

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ABSTRACT

In present study a sustainable greener method is used to synthesise 1, 3, 4-thiadiazole derivative of Guargum. Thiadiazole nucleus is an integral part of various medicines. Guargum is a non-ionic polysaccharide commonly used in oil, paints and pigments, cosmetic and food industries etc. The reaction was carried out in Microwave. The newly synthesized derivative was characterized by FTIR-spectroscopy, H^1 NMR, Mass spectrometry and elemental analysis is done by khjeldhal method. Antimicrobial activities were studied on different strains using well diffusion method.

Keywords: Guargum, 1, 3, 4-thiadiazole, microwave assisted synthesis, antimicrobial activity.

INTRODUCTION

Despite the vast importance of biopolymer there is a need to modify synthetic non degradable polymeric product to biodegradable polymeric product. Guar gum is a potential aspirant of naturally occurring biodegradable polymer due to its non-toxicity, biodegradability, biocompatibility, stability over wide pH range and modifying rheological properties.[1-3] Guar gum (*Cymopsistetra gonoloba*) represents Galactomannan family of polysaccharides. Although this is easily available at low cost but its uncontrolled hydration upon storage and further microbial contamination limits its long term application. Chemical modification of Guar gum diversifies and enhances its applications and functionality. In present study this polysaccharides is derivatized by thiadiazole nucleus using microwave irradiation.

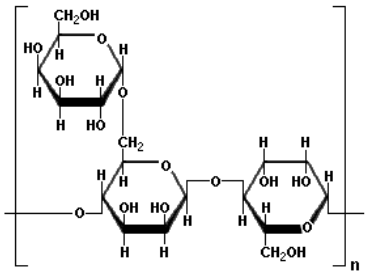

Figure 1: Guar gum structure

Thiadiazole is an integral part of many natural products and medicinal agents. It shows potential biological activities.[4-5] Among several isomers of thiadiazole moiety 1,3,4-thiadiazole isomer is extensively studied.[6] Its derivatives possess significant antimicrobial,[8] anti-convulsant[9], antidiabetic,[10-11] anti-depressant[12] and anti-cancer activities[13-14].Byincorporating thiadiazole nucleus to Guar gum we got a novel derivative which has more significant antimicrobial activity than its parent compound.

Now a day's Microwave assisted green synthesis has been proved as an efficient protocol due to faster reactions, lesser by products, purity of compounds, absolute control over reaction parameters and higher yield as compared to conventional method.[15]

MATERIALS

Guar (200 mesh size) was procured from local industry. All AR grade chemicals used were procured from Sigma Aldrich, Loba Chemicals, and Ases chemical works. The bacterial and fungal strains used for evaluation of antimicrobial activities, were obtained from S.N. Medical College, Jodhpur.

METHODS

Synthesis of epoxy ether of Guar

1 mole of guaran powder was slurried in DMSO solvent in a round bottom flask. Then 50% aqueous NaOH was added in the slurry to make the reaction mixture alkaline, and the mixture was constantly magnetically stirred at 45°C for 2 hours. Further 1 mole of epichlorohydrin was added gradually with continuous stirring and the pH was adjusted to 9-10 then this reaction mixture was subjected to microwave for 15 minutes. Later, the compound was filtered on vacuum pump with 80% aqueous methanol containing few drops of nitric acid to remove inorganic impurities of chloride ion and excess of alkali. (Fig-2)

Synthesis of 5-(aryl amino)- 2-sulfanyl 1,3,4- thiadiazole

0.1 mole of aniline was dissolved in 20 ml of ammonia solution to which 0.1 mole of carbon disulphide was gradually added with constant stirring. The temperature of the solution was kept below 30°C.20-25 ml of ethanol was then added and the stirring was continued till all the carbon disulphide dissolved. The reaction mixture was then allowed to stand for 2 hrs.

Equimolar quantity of sodium hydroxide and monochloroacetic acid were taken, dissolved separately in water and cooled. After cooling, the solutions were mixed to obtain sodium salt. This sodium chloroacetate solution was added to the reaction mixture followed by the addition of 10 ml of 50% hydrazine hydrate. The mixture became warm that was cooled. After cooling it was filtered, dried and recrystallized with ethanol.[18] (Fig-3)

Synthesis of thiadiazole derivative of Guar Gum

1,3,4-thiadiazole and Chloro acetyl chloride were taken in equimolar ratio in a beaker and 20-25 ml Ammonia solution is added. The mixture was then stirred and 4-5 ml of formaldehyde and 6 ml Ammonia solution were added to the reaction mixture. 20-25 ml DMSO was added. This mixture was irradiated for 10-12 minutes in Microwave.

Now to this reaction mixture 1,2- epoxy propyl guar is added and again the mixture was subjected to microwave irradiation for 10-12 minutes. The so formed derivative was filtered and purified with ethanol.(fig-4)

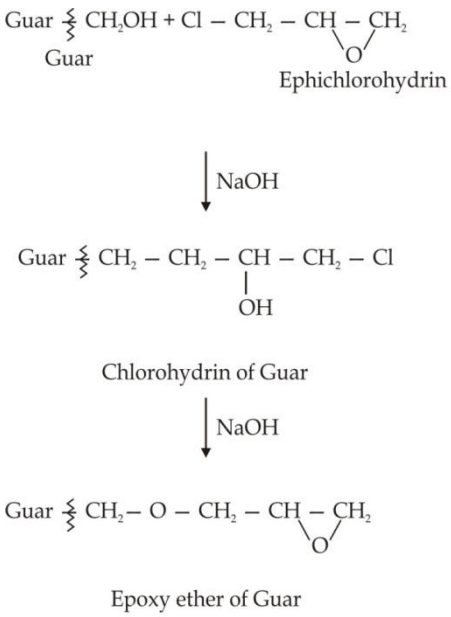

Figure 2: Synthesis of epoxy ether of Guar

Characterization

Melting point of the compound is determined in open capillary tube and is uncorrected. The newly formed derivative was characterized by FTIR spectroscopy, H^1 NMR Spectroscopy, Mass spectrometry, elemental analysis.

Antimicrobial Activity

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan. Thiadiazole is an important heterocyclic nucleus and it has occupied a pivotal position in medicinal chemistry because it is having a broad spectrum of pharmacological activities especially potent antimicrobial activity against a wide variety of microbes like bacteria and fungi.[16]

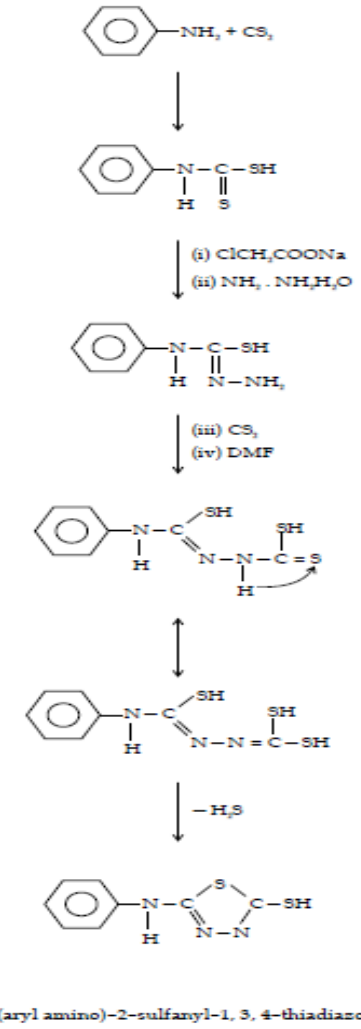

Figure 3: Synthesis of 5-(aryl amino)- 2-sulfanyl 1,3,4-thiadiazol

Minimum inhibitory concentration (MIC) of synthesized derivative for bacteria was determined against 2 gram positive and 2 gram negative bacterial strains by broth dilution method. Ampicillin (500µg/ml) was used as standard drug. MIC for antifungal activity was carried out against *Candida albicans* and *C.tropicalis* and the results were compared with standard drug fluconazole (100µg/ml) by same method.

Preparation of inoculums

Pure isolate of each bacterium and fungi are first sub cultured in nutrient broth at 37°C for 24h.The plates were inoculated by dipping a sterile swab into inoculums. The swab was streaked all over the surface of the medium (MHA for bacteria and MHA-GMB for fungi) three times, rotating the plate through an angle of 60° after each application. Finally the swab was passed round the edge of the agar surface. The inoculums were dried for a few minutes, at room temperature, with the lid closed.

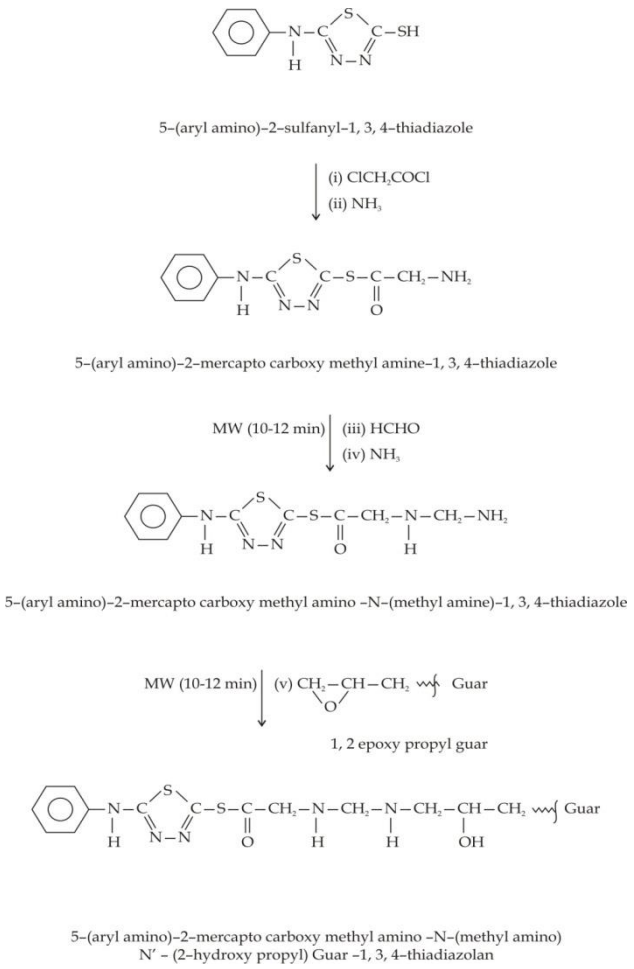

Figure 4: Synthesis of thiadiazole derivative of Guar Gum

Antimicrobial susceptibility test

Wells of approx 6 mm were dig on the sterile agar plate. MIC of test compound in DMSO was filled in well using micropipette. A control was also prepared using DMSO. The plates were incubated. If the bacterial or fungal strain were susceptible to the antimicrobial agent then a zone of inhibition was obtained on agar plate. This zone of inhibition was measured by transparent ruler and results were recorded.

RESULTS & DISCUSSION

Successful microwave assisted synthesis of Guar thiadiazole derivative was done without using any catalyst. Reaction results concluded greater product yield (80%) as compared to yield (68%) obtained by conventional heating method. Nitrogen content of the compound is 18%.

FT-IR Analysis

IR Spectra was recorded with BRUKER spectrophotometer. The spectrum of the newly synthesized compound shows a peak at 961cm^{-1} for C=S stretching. Peaks at 1547.86 cm^{-1} and 1691.54 cm^{-1} correspond to C≡N stretching and CH_2 bending vibrations respectively. The bands at 3500 cm^{-1} and 3740.68 cm^{-1} attribute to NH_2 stretching and O-H group respectively.(fig 5)

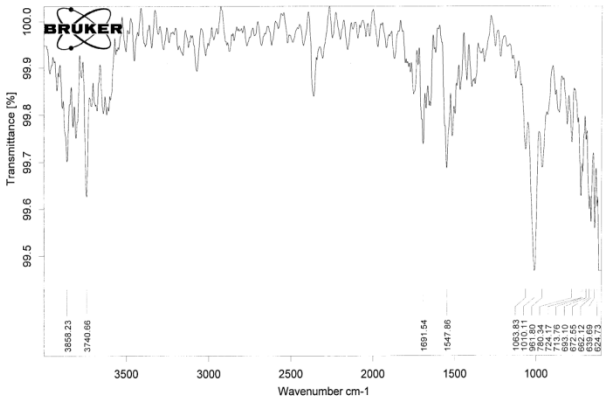

Figure 5: IR Spectra

H^1 NMR Analysis: NMR

A spectrum was determined by Bruker AV-II 300 MHz FT-NMR Spectrometer. The compound was dissolved in DMSO. The interpretation shows peak at δ3.5 for aromatic amine proton (C_6H_5N-H). Other peaks at δ2.56 are due to CH_2 and δ7.2 is due to aromatic proton (Ar-H). (fig-6)

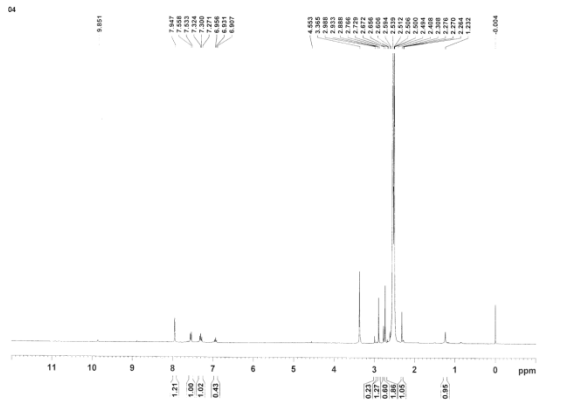

Figure 6: H^1 NMR Analysis

Mass spectral Analysis

DART-MS was recorded on a JEOL-Accu TOF JMS-T1OOLC Mass spectrometer having a DART (*Direct analysis in real time*) source. The compound was subjected as such in front of DART source. Dry Helium was used with 4 LPM flow rate for ionization at 350°C. The orifice 1 was set at 28 V. Mass spectral analysis- Base peak at 157.07(fig-7)

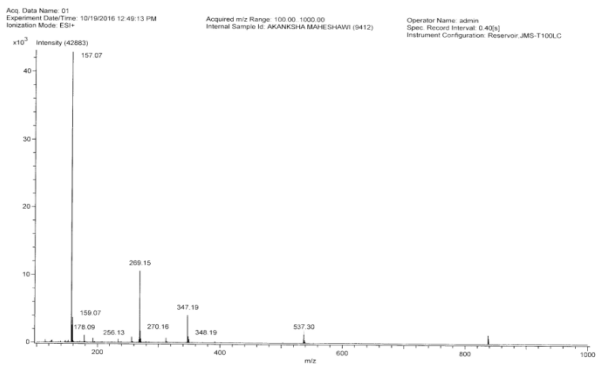

Figure 7: Mass spectra

Antimicrobial Activity

The antimicrobial evaluation of the newly synthesized Guar derivative states that the compound exhibits significant activity against gram negative bacteria and moderate activity towards gram positive bacteria(Table 1). It shows inhibition zone against *Escherichiacoli, klebsiella pneumonia, Staphylococcus aureus* and no zone of inhibition against *Pseudomonas aeruginosa.* Fungal strains like C*andida albicans, Candida tropicalis* do not grow in presence of the compound (Table 2).

Table 1: Anti-bacterial activity of 1, 3, 4-thiadiazole derivative of Guar gum

S. No.	Bacterial strains	Type	Zone of Inhibition	
			250µg/ml	*500µg/ml*
1.	***Escherichia coli***	Gram Negative	10mm	18mm
2.	***klebsiella pneumonia***	Gram Negative	11mm	19mm
3.	***Staphylococcus aureus***	Gram Positive	7mm	12mm
4.	***Pseudomonas aeruginosa***	Gram Positive	No	No

Table 2: Antifungal activity of 1, 3, 4 thiadiazole derivative of guargum

S.No.	Fungal Strains	Zone of Inhibition	
		100µg/ml	*200µg/ml*
1.	***Candida albicans***	6mm	14mm
2.	**Candida tropicalis**	5mm	12mm

CONCLUSION

The properties of microwave synthesized derivative are superior to the derivative which synthesized conventionally. Without having compromise on energy efficiency or yield of the product this synthetic technique works towards achieving the goal of cleaner technologies. The synthesized compound showed significant antimicrobial activity against gram positive and moderate activity against gram negative bacteria and fungi. So it is concluded that there exists ample scope for further study in this class of compounds.

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