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Synthesis and Antimicrobial Evaluation of Bio-Based Polyol Derived from Chia Seed Oil for Sustainable Polymer Applications

A. Jeni[1], S.Begila David[2]
[1,2]Research scholar, Department of Chemistry, 21213162032020, Scott Christian College, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, TamilNadu, India.
jenibino5@gmail.com
*Corresponding Author, Associate Professor, Department of Chemistry, Scott Christian College, Nagercoil-629003, TamilNadu, India.
begilarobin@gmail.com

ABSTRACT:
Research on bio-based polymers made from renewable resources has intensified due to the rising need for sustainable products. The oil from chia seeds (Salvia hispanica L.) was used in this work as a precursor for the sequential epoxidation and hydroxylation that produced a multifunctional polyol. FTIR and NMR spectroscopy were used to validate the structural change, exposing distinctive hydroxyl and ester functionalities. The polyol's phytochemical screening revealed the presence of bioactive substances such reducing sugars, saponins, and phenolics. When compared to raw chia seed oil, antibacterial testing showed larger inhibition zones for the polyol against Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli, indicating greater antimicrobial activity after functionalisation.These findings highlight the potential of polyol produced from chia seeds as a bioactive and sustainable monomer for the synthesis of polyester, with potential uses in biodegradable packaging and antimicrobial coatings.
Keywords:*-Chia seed oil , Bio-based polyol Epoxidation, Hydroxylation, FTIR spectroscopy, Phytochemical screening , Antibacterial activity.*

INTRODUCTION

The strong demand for petrochemical-derived products and their harmful impacts on the The environment, alongside the increasing scarcity of these non-renewable resources, are factors that have prompted the chemical industry to seek out new sources of renewable materials as raw inputs. These raw inputs have significantly aided the sustainable advancement of the plastics sector, thanks to nature's immense synthetic potential and various green principles. chemistry [1,2]. Vegetable oils rank among the most regarded alternatives because of their availability, minimal toxicity, ecological safety, natural fluidity, and economical pricing [3–5]. Numerous vegetable oil molecules need to undergo chemical transformation to produce polyols, which are utilized for the creation of polyurethanes [6], polyesters [7], and epoxy [8], among other materials. Numerous studies have concentrated on the production of bio-polyols from fatty acids and plant oils. It is essential to understand that vegetable oils, apart from castor and lesquerella oils, lack hydroxyl groups.

Consequently, it is essential to alter the plant chemically. oils to incorporate hydroxyl groups into their structures to create polyols. Five distinct methods exist for producing vegetable oil-based bio-polyols: (1) epoxidation followed by oxirane ring-opening [9]; (2) hydroformylation in conjunction with hydrogenation [10,11]; (3) ozonolysis [12]; (4) thiol–ene coupling [13]; and (5) transesterification or amidation [14]. The initial approach is the most commonly utilized. This method is frequently employed by researchers in either one or two phases. "The one-step process involves in situ epoxidation, followed by hydroxylation with acetic acid, sulfuric acid, and hydrogen peroxide." The two-stage process involves the epoxidation of triglycerides, succeeded by the ring-opening of oxirane, utilizing difunctional compounds like alcohols or amines" [15,16].

Renewable resources including starch, lignin, protein, cellulose, chitosan, shellac, rosin, polyhydroxyalkanoates, furanone, alginate, wool fibres, and vegetable oils [VO] are the sources of polymers. Plasticisers, biodiesel, lubricants, adhesives, biodegradable packaging materials, printing inks, paints, and coatings are just a few of the countless industrial uses for them. VO is a domestically plentiful, non-toxic, non-depletable, non-volatile, and biodegradable resource. They produce polymers that can rival

petroleum-based goods made from fossil fuels. In addition to their extensive industrial uses, these polymers are used in the creation of paints and coatings (Dutton and Scholfield, 1963; Wisniak, 1977; Baumann et al., 1988; Schuchardt et al., 1998; Lu and Larock, 2009; Xia and Larock, 2010; Salimon et al., 2012).Even in the era of cave art, VO was the main ingredient in paints and coatings. Today, polymer chemists and technologists have returned to the widespread use of VO derived materials in paints and coatings because of the numerous environmental and health risks associated with products derived from fossil fuels, as well as concerns about the depletion of petroleum resources by the end of the twenty-first century.

Historical documents indicate that ancient Mesoamerican cultures—such as the Aztecs and Mayas—utilized Salvia hispanica L. alongside corn, beans, and amaranth in creating traditional medicines and meals. In pre-Columbian cultures, it was the second most important crop following beans. In Aztec societies, chia served as a food source, a cosmetic ingredient, and for religious ceremonies.

In recent years, Chia seeds have emerged as one of the globe's most well-known foods due to their dietary benefits and healing qualities [3,5,6,7,10]. Coorey et al. [11] noted that Chia is a remarkable ingredient because it has the highest known concentration of α-linolenic acid and can be conveniently incorporated into commercial food. Numerous studies have indicated that chia seeds—owing to their significant fatty acid content—can be essential for health, providing antioxidant and antimicrobial benefits [3,6,12,13,14]. Recently, many new findings have emerged concerning the nutritional attributes, phytochemicals, and extraction techniques related to chia seeds.

MATERIALS AND METHODS

Chia seeds were purchased from the local market Nagercoil. Sample's were stored in polythene bags at 4°C until processing. The seeds of salvia hispanica were individually washed with double distilled water and were left to dry at 40°C for 2 days. The dried seeds were grind well in to a fine powder with a mixer cylinder respectively. The powder was stored in air sealed plastic containers at room temperature till Extraction was carried out.

Preparation of Powdered Extract

The oil Extraction was carried out using AOAC technique Am2-93 (official methods and recommended practices of the American oil chemists society ; AOCS press : champaign , IL, USA, 1995).

The soxhlet apparatus was used to extract the chia seed oil uing hexane as a solvent. First , 10g of chia seeds powder were packed in to a polythene bags, which wa placed in the soxhlet apparatus. Next , 100ml of hexane was added to the round bottom flask, and the apparatus wa s heated using a heating mantle . the hexane solvent was allowed to reflux through the soxhlet apparatus for 6 - 8 hours , during which time the chia seed oil was extracted in to te hexane solvent.

Finally, the hexane solvent was removed from the extract using rotary vacuum evaporator, and the chia seed oil was stored in the refrigerator till additional research was conducted.

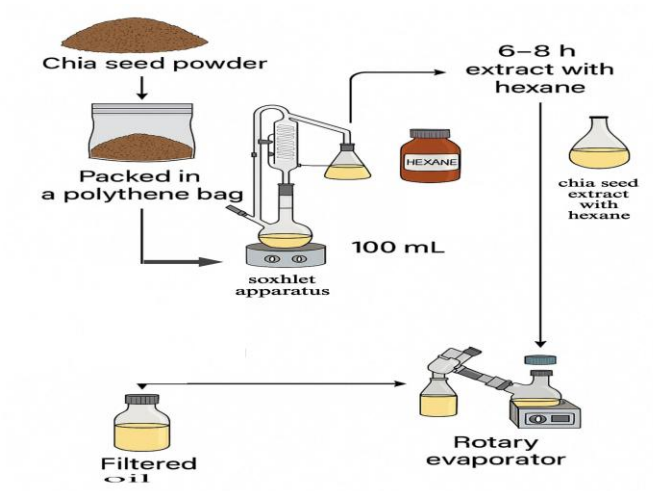


Fig:-1 Schematic representation of Extraction of Chia Seed oil from Chia seed

SYNTHESIS OF POLYOL:

Epoxidation:-

50 ml of glacial acetic acid CH3COOH was introduced to the reactor along with 30ml of 30% H2O2 . After that 2ml of 96% H2SO4 was added and the mixture was agitated for an hour at 200rpm at 40°c. After three hours of stirring at 200rpm at 40°c, 100 ml of Chia seed oil was added, allowed to cool to room temperature, and the oil phase was separated.

Hydroxylation:-

100 of methanol, 50ml glycerol 2 ml of 96%H2SO4 and 5ml of deionized water was placed in to a 250ml three-necked flask and heated to 40°c, then the Oxidized oil was added and agitated for 25 hours at 50°C and cooled to room temperature. The resulting polyol was further more Characterized.

Identification of Phytochemical Screening of Chia Seed Oil

Phytochemical Screening of Chia Seed Oil (Salvia Hispanica Oil)

Widely adopted precipitation and colorimetric methods were employed to identify both primary and secondary metabolites through qualitative phytochemical screening and basic radical identification. These procedures were conducted to evaluate the chemical composition of various crude extract samples. Specifically, phytochemical tests were performed on the aqueous extract of chia seed oil. Approximately 10 g of air-dried chia seed oil extract was carefully extracted using distilled water. The resulting extract was subjected to standard phytochemical assays to detect the presence of steroids, alkaloids, reducing sugars, phenolic compounds, saponins, carbohydrates, flavonoids, tannins, lipids, anthraquinones, and amino acids.

Identification of Basic Radicals

The preliminary identification of basic radicals was carried out following standard qualitative inorganic analysis protocols (Vogel, 2005). Approximately 2 g of chia seed oil was first subjected to saponification with alcoholic KOH to obtain the aqueous soap solution containing soluble salts. The resulting solution was acidified and used for systematic radical analysis. Group separation was performed using classical group reagents such as dilute HCl (Group I cations), H_2S in acidic medium (Group II), NH_4OH/NH_4Cl buffer (Group III), $(NH_4)_2CO_3$ (Group IV), and Na_2HPO_4 (Group V). Characteristic confirmatory tests were carried out for individual cations such as Pb^{2+}, Cu^{2+}, Fe^{3+}, Zn^{2+}, Ca^{2+}, and Mg^{2+} by their respective color reactions, precipitate formation, or flame tests. Observations were compared with standard test results to confirm the presence or absence of basic radicals in the chia seed oil extract.

FTIR ANALYSIS:

Using a Shimadzu FTIR-8400S spectrophotometer, FTIR was used to determine the chemical structure of the SHO and SHP. The wavelength range in which the spectra were observed was 500–4000 cm-1.

NMR SPECTROSCOPY

^1H and ^{13}C NMR spectra of the synthesized polyol were recorded on a Bruker Avance III 400 MHz spectrometer at room temperature. About 10 mg of sample was dissolved in 0.7 mL DMSO-d_6, using TMS as the internal reference. Spectra were processed with TopSpin 3.6 software. Characteristic signals for aliphatic –CH_2–/–CH– protons, hydroxyl groups, and ester carbonyl carbons confirmed the successful formation of the polyol.

ANTIBACTERIAL ACTIVITY

The Kirby–Bauer disk diffusion method was used to determine antimicrobial susceptibility. Mueller–Hinton agar was prepared according to standard guidelines and poured into sterile Petri plates to a uniform depth of 4 mm. Test organisms were suspended in sterile saline and adjusted to a 0.5 McFarland standard, followed by uniform inoculation of the agar surface using a sterile swab. Commercially prepared antibiotic discs were aseptically placed on the inoculated plates and gently pressed to ensure complete contact with the agar surface. Plates were incubated inverted at 37 °C for 16–18 h, and the diameter of inhibition zones, including the disc, was measured in millimeters to interpret susceptibility.

RESULT AND DISCUSSION

Phytochemical screening Of Salvia Hispanica Oil (SHO)

EXPERIMENT	OBSERVATION	INFERENCE
NaOH test SHO Extract + 2ml of NaOH	No blue green color obtained	Absence of anthocyanin
Bromine water test: SHO Extract + bromine water	Pale yellow color obtained	**Presence of glycosides**
1ml of + lead acetate solution	No precipitate obtained	Absence of phenol
5 ml of SHO extract + 2ml of CHCl3 + 3 ml of conc. H2SO4	No reddish-brown precipitate obtained	Absence of terpenoids
LEAD ACETATE TEST: SHO Extract + 1ml of lead acetate	No red precipitate	Absence of tannins
SHO Extract + CHCl3 + conc. H2SO4	No purple color obtained	Absence of steroids
SHO Extract + Molisch's reagent	Purple color is obtained	**Presence of reducing sugars**
SHO Extract + 2N HCl and remove the aqueous layer and add Mayer's reagent	No white precipitate obtained	Absence of alkaloids
Alcohol + SHO extract + ferric chloride	Intense color	**Presence of phenolic compounds**
SHO Extract + water + shake well	Foamy lather is obtained	**Presence of saponins**
Alcohol + SHO extract + Mg ribbon + Conc. HCl	No color change	Absence of flavonoids
Alcohol + Conc. HNO3 + NH3+ SHO Extract	No reddish orange color is obtained	Absence of xanthoproteins

Basic radicals Identification of SHO

EXPERIMENT	OBSERVATIONS	INFERENCE
SHO Extract + KI	No golden spangles	Absence of lead
SHO Extract + NH4OH	white precipitate	**Presence of bismuth**
SHO Extract + cupron reagent + NaOH	No green color obtained	Absence of copper
SHO Extract + potassium ferrocyanide	No white precipitate obtained	Absence of zinc
SHO Extract + dil. HCl + water + H2S	No yellow precipitate	Absence of cadmium
SHO Extract + potassium thiocyanate	No red or blue color	Absence of ferric and cobalt
SHO Extract + dil. HCl + aluminon reagent + (NH4)2CO3	No bright red precipitate is obtained	Absence of aluminum
SHO Extract + conc. HNO3 + sodium bismuthate + water	No pink color	Absence of manganese
SHO Extract + dimethyl glyoxime + NH4OH	No scarlet red precipitate	Absence of nickel
SHO Extract + potassium chromate	Pale yellow precipitate	**Presence of barium**
SHO Extract + NH4OH + ammonium oxalate	White precipitate	**Presence of calcium**
SHO Extract + Magneson reagent + NaOH	Blue precipitate	**Presence of magnesium**
SHO Extract +NaOH + Nesseler's reagent	No reddish-brown precipitate	Absence of ammonium

Structural Characterization by NMR

The 1H NMR spectrum of the chia seed–based polyol exhibited a singlet at δ 5.69 ppm, which may correspond to hydroxyl or residual olefinic protons. A broad multiplet observed between δ 3.3–4.8 ppm is attributed to protons on carbon atoms adjacent to hydroxyl groups (–CH_2–OH, –CH–OH), confirming the hydroxylation of the triglyceride backbone. Additional signals appearing at δ 2.56 ppm and 1.87 ppm are consistent with methylene groups adjacent to ester or hydroxyl moieties, while the peaks at δ 1.10 and 0.96 ppm are assigned to terminal methyl groups, likely originating from the fatty acid chains of the original chia seed extract.

The corresponding 13C NMR spectrum revealed multiple signals between δ 61.43 and 73.55 ppm, indicating the presence of both primary and secondary hydroxyl-bearing carbon atoms. Notably, the signal at δ 69.40 ppm is indicative of a central carbon in a glycerol-like or triol structure, while the signals at δ 65.79, 62.86, and 61.43 ppm confirm the presence of hydroxymethylene groups. These data confirm successful hydroxylation and transformation of the fatty acid-derived components into a multifunctional polyol structure.

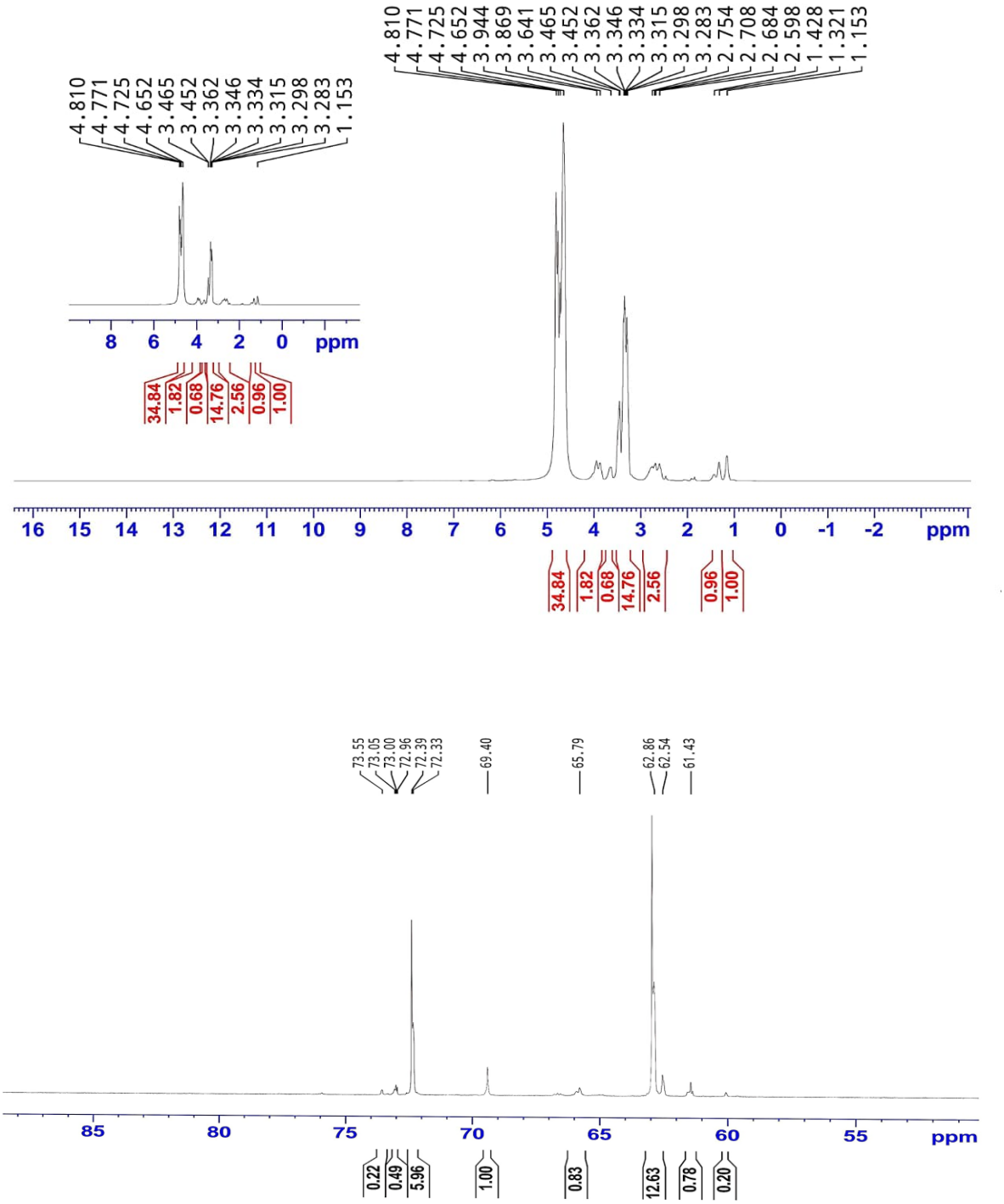


Fig:2 **NMR Spectroscopy for chia seed based polyol**

Upon polymerization with a diacid or diester, the 1H NMR spectrum of the resulting bio-based polyester showed a notable decrease in hydroxyl-related signals, along with the appearance of new resonances in the δ 4.0–4.3 ppm region. These are characteristic of methylene protons adjacent to ester linkages (–CH_2–O–CO–). The persistence of aliphatic signals between δ 1.2 and 2.5 ppm indicates that the polyol backbone was retained in the polymer chain, imparting flexibility and hydrophobic character.
Although the 13C NMR spectrum of the polyester (not fully shown here) would be expected to exhibit new signals between δ 165–175 ppm corresponding to ester carbonyl carbons, the preserved signals in the δ 60–74 ppm region confirm that the polyol structure remains chemically integrated within the final polyester network.

Fourier Transform Infrared (FTIR) Spectroscopy

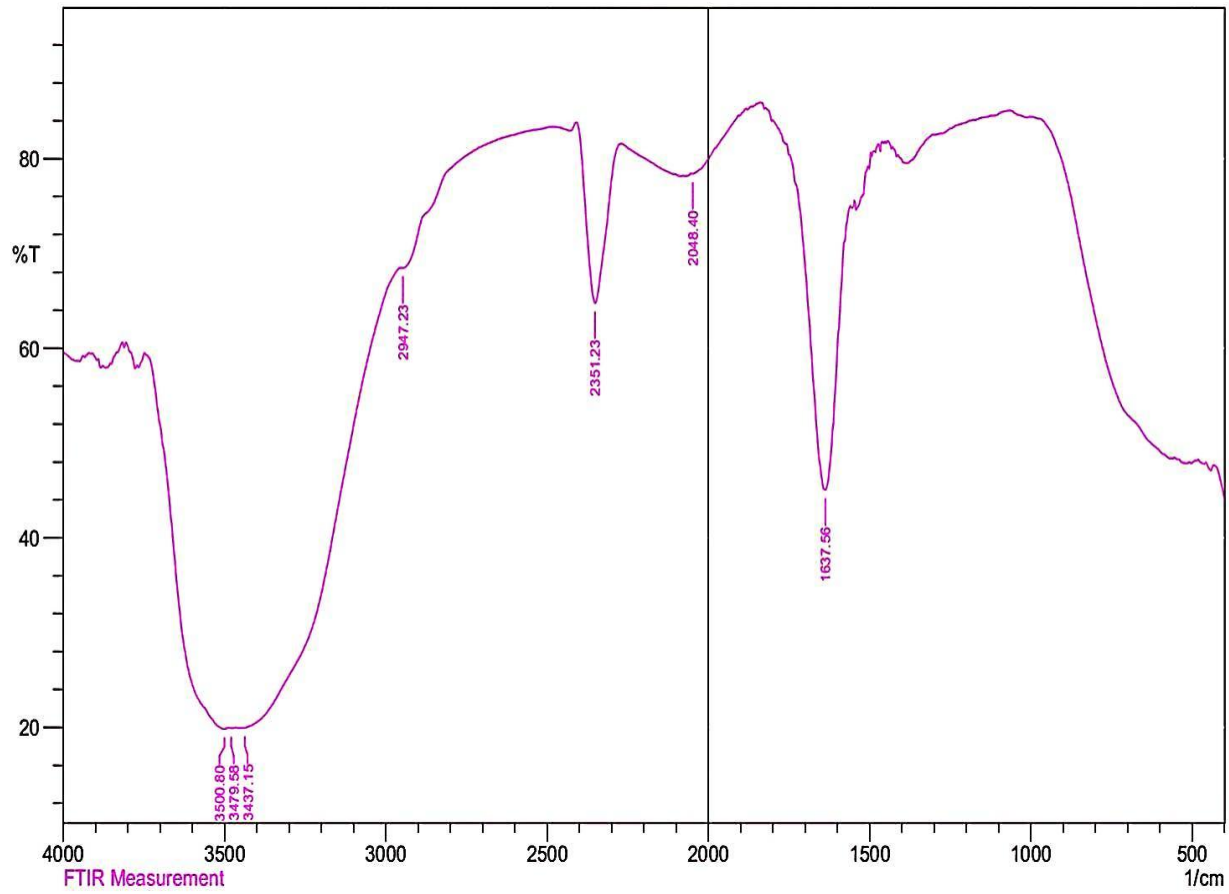


Fig:-3 FT-IR spectra of Bio-Based Polyol

FTIR spectroscopy was employed to confirm the presence of functional groups in the synthesized polyol. The spectrum was recorded in the range of 4000-500 cm-1 using a Shimadzu FTIR-8400S spectrometer (Figure 3) The FTIR spectrum of the synthesized polyol exhibited a broad O–H stretching band at 3500.8 cm^{-1} and 3479.58 cm^{-1}, confirming the presence of hydroxyl groups characteristic of polyol functionality. A hydrogen-bonded O–H stretch was observed at 3437.15 cm^{-1}. The strong peak at 2947.23 cm^{-1} corresponds to C–H asymmetric stretching of $-CH_2$ and $-CH_3$ groups. Minor bands at 2515.23 cm^{-1} and 2048.4 cm^{-1} are attributed to overtone or combination bands, possibly due to CO_2 interference or instrument artifacts. A distinct peak at 1637.56 cm^{-1} indicates residual C=C stretching or H–O–H bending, suggesting the presence of trace unsaturation in the sample.

The addition of hydroxyl groups to the polyol is confirmed by the wide O–H absorption at 3500–3479 cm^{-1} in the FTIR spectra of chia seed polyol. At 2947 cm^{-1}, a prominent C–H stretching band suggests the existence of aliphatic chains made from chia seed oil. The modest absorption at 1637 cm^{-1} indicates incomplete hydrogenation and is consistent with residual C=C bonds. Overtone vibrations or ambient CO_2 might be the cause of additional combination bands at 2515–2048 cm^{-1}. All things considered, our findings validate that chia seed oil was successfully functionalised with hydroxyl to produce polyol.

Antibacterial Activity of Chia Seed based Polyol and Chia Seed Oil

The antibacterial activity of chia seed polyol (sample A) and chia seed oil (sample B) was evaluated against the four test bacteria using the Kirby–Bauer disk diffusion method (Fig. 3a–d). The inhibition zones are summarized in Table 3

Antibacterial Activity of Chia Seed based Polyol and Chia Seed Oil

Test organism	Polyol [A] Zone of inhibition [mm]	Chia seed oil [B] Zone of inhibition [mm]	Standard antibiotic Cefotaxime Zone of inhibition [mm]
E.coli	10	6	22
Klebsiella	12	4	20
Bacillus	5	6	20
Staphylococcus aureus	12	8	22

The chia seed polyol exhibited moderate antibacterial activity, with inhibition zones ranging from 5 mm (Bacillus sp.) to 12 mm (K. pneumoniae and S. aureus). Polyol-treated discs showed clear inhibition halos, suggesting that chemical hydroxylation enhanced the antimicrobial properties of chia seed oil.
Chia seed oil alone showed lower antimicrobial activity, with inhibition zones between 4–8 mm. The highest inhibition was against S. aureus (8 mm), while K. pneumoniae showed minimal sensitivity (4 mm).

The standard antibiotic cefotaxime consistently produced large inhibition zones (20–22 mm) against all strains, validating assay performance and providing a benchmark for comparison.
Importantly, the polyol demonstrated enhanced antibacterial efficacy compared to chia seed oil for three out of four bacterial strains (E. coli, Klebsiella, and S. aureus). This improvement may be due to the introduction of hydroxyl groups, which can increase polarity and facilitate interactions with bacterial membranes or enzymes, potentially disrupting cellular function.

While the inhibition zones of the polyol were lower than those of cefotaxime, the observed antibacterial activity indicates potential dual functionality: (i) as a sustainable bio-based precursor for polyester synthesis and (ii) as an additive with mild antimicrobial properties, useful in coatings, packaging, and other green material applications.

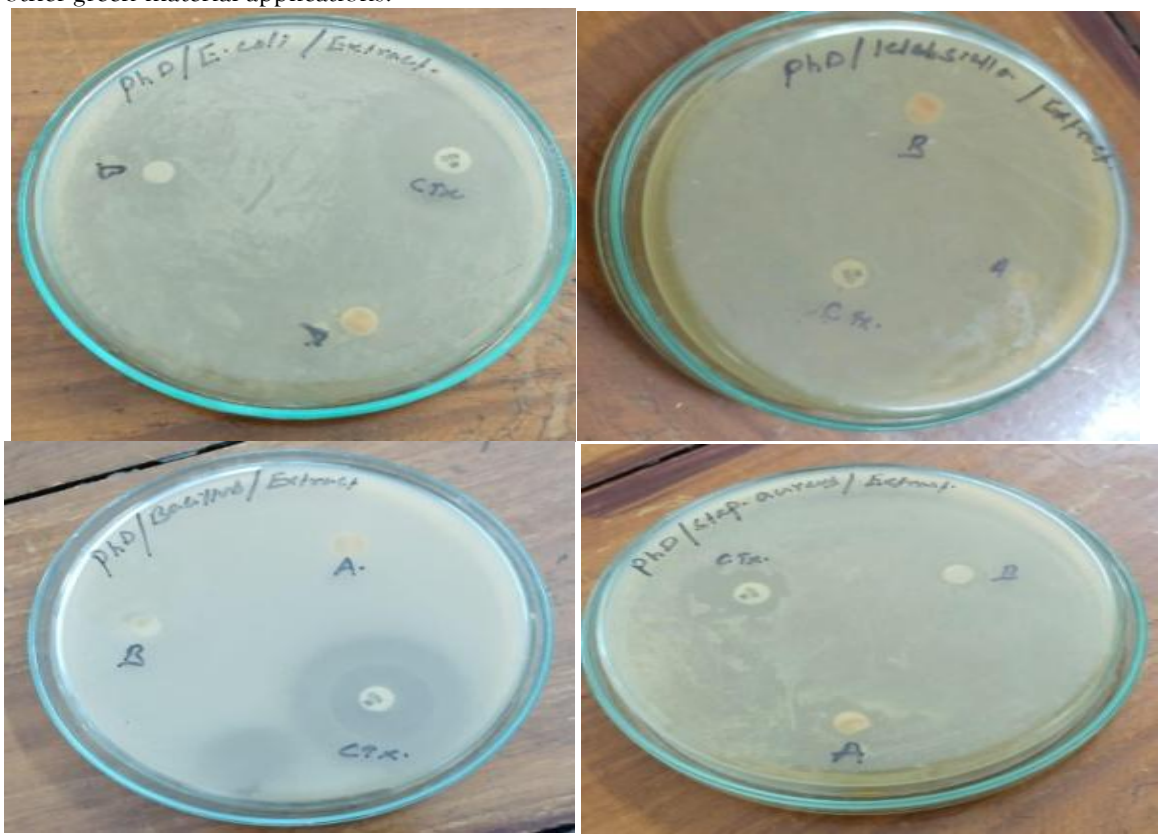
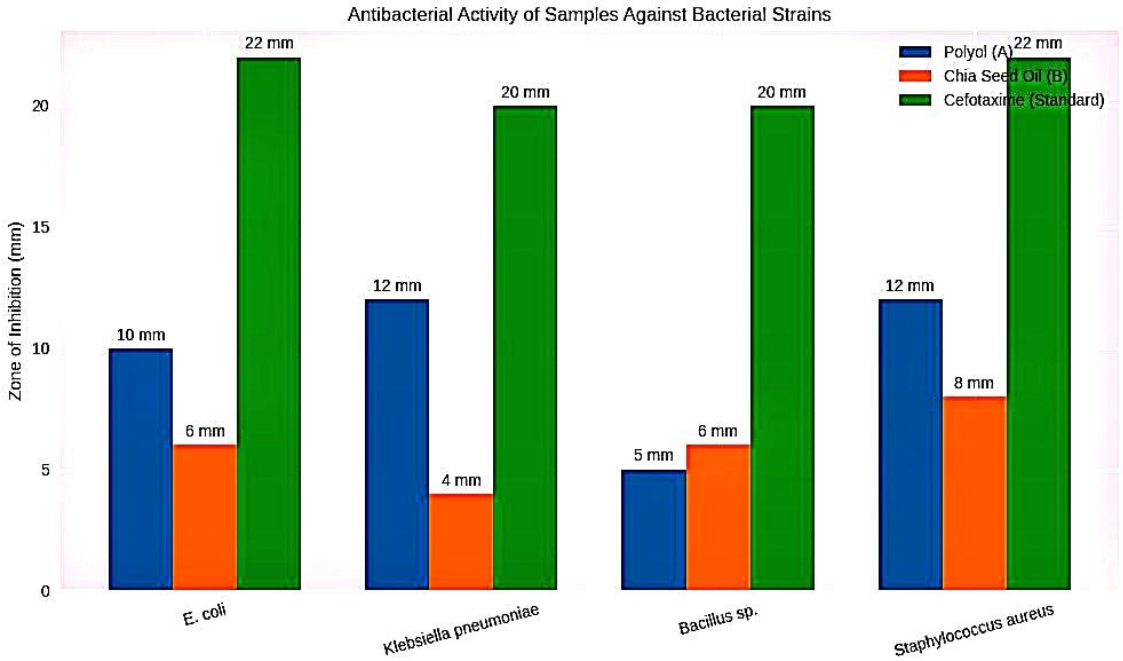


Fig:6Antibacterial Activity of Chia Seed based Polyol and Chia Seed Oil

comparing the antibacterial activity of Polyol (A), Chia Seed Oil (B), and Cefotaxime (Standard) against four bacterial strains. Each bar represents the zone of inhibition in millimeters, giving a clear visual of how each sample performed.



Polyol (A) shows notably higher activity than oil, especially against Klebsiella pneumoniae and Staphylococcus aureus.Cefotaxime remains the most potent across all strains. Bacillus sp. appears least susceptible to both polyol and oil.

Antifungal Activity of Chia Seed based Polyol

The antifungal activity of chia seed–based polyol was assessed using the Kirby–Bauer disk diffusion method against Candida albicans and Aspergillus flavus. Nystatin served as the standard control. The inhibition zones around the disks were measured in millimeters (mm), and the results are presented in Table 4

Antifungal Activity of Chia Seed based Polyol

Test organism	Polyol (mm)	Control (mm)
C.albicans	15mm	17mm
Aspergillus flavus	16.3mm	15mm

The chia seed–based polyol demonstrated measurable antifungal activity against both test organisms. Against Candida albicans, the polyol produced an inhibition zone of 15 mm, which was slightly lower than that of the control, nystatin (17 mm). In contrast, against Aspergillus flavus, the polyol exhibited a larger inhibition zone (16.3 mm) compared to nystatin (15 mm).

The results confirm that the chia seed–based polyol exhibits broad-spectrum antifungal properties, showing promising inhibition against both yeast (Candida albicans) and filamentous fungi (Aspergillus flavus). The higher inhibition against Aspergillus flavus suggests that the polyol is particularly effective against molds, potentially disrupting fungal cell walls or interfering with spore germination.

The antifungal effect can be attributed to bioactive constituents derived from chia seed oil, including phenolic compounds and hydroxyl groups introduced during the polyol synthesis. These functional groups may disrupt fungal membrane integrity, increase permeability, and lead to growth inhibition.

The comparable performance to nystatin—and even superior inhibition in the case of Aspergillus flavus—highlights the potential of chia seed–based polyol as a natural, bio-derived antifungal agent for

applications in pharmaceutical formulations, medical coatings, and biodegradable materials where fungal resistance is desirable.

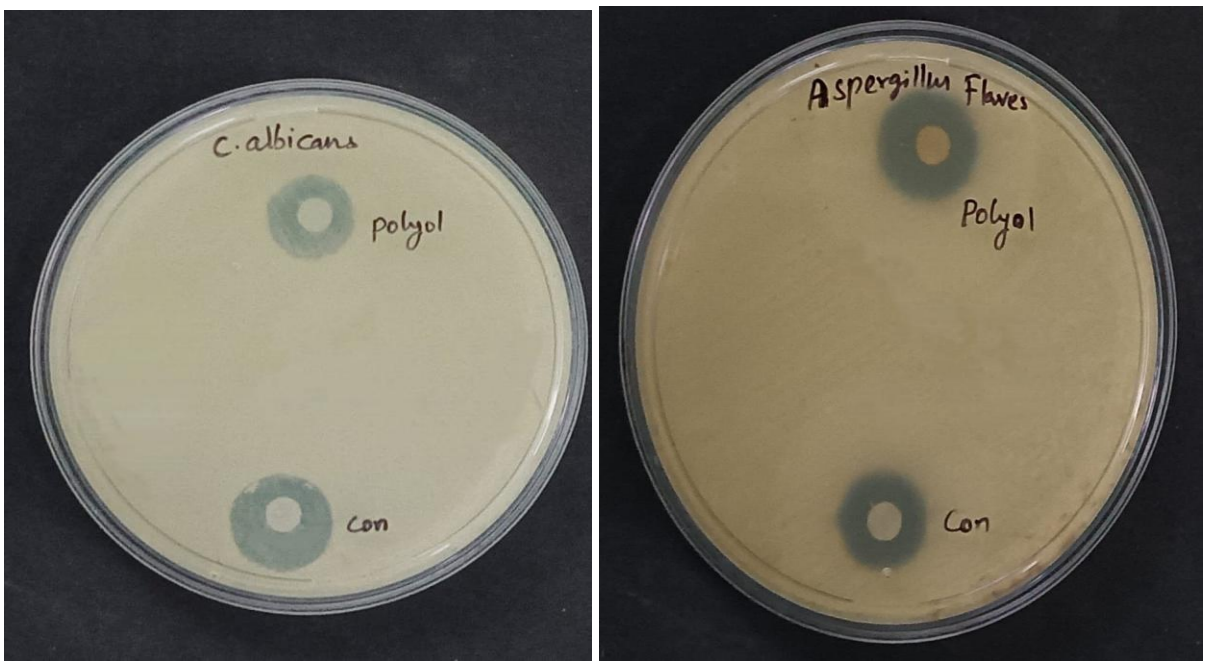


Fig:- 5 Antifungal activity of chia seed based polyol

CONCLUSION:-

By using epoxidation and hydroxylation to successfully synthesise a bio-based polyol from chia seed oil, this work produces a structurally altered molecule with improved functional qualities. The addition of hydroxyl groups was verified by spectroscopic investigations (FTIR and NMR), and phytochemical screening showed that bioactive components such as phenolics, saponins, and reducing sugars were retained. Compared to unmodified chia seed oil, the polyol showed higher antibacterial activity against Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli, indicating that chemical functionalisation greatly increases its antimicrobial ability. The present study successfully synthesized a bio-based polyol from chia seed oil through epoxidation and hydroxylation, resulting in a structurally modified molecule with improved functional properties. Spectroscopic analysis (FTIR and NMR) confirmed the incorporation of hydroxyl groups, while phytochemical screening revealed the retention of bioactive compounds such as phenolics, saponins, and reducing sugars. The synthesized polyol demonstrated enhanced antibacterial and antifungal activities compared to unmodified chia seed oil, showing notable inhibition against Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Candida albicans, and Aspergillus flavus. These findings highlight the dual potential of chia seed–based polyol as both a sustainable monomer for polymer synthesis and a bioactive material for antimicrobial applications, making it a promising candidate for use in biodegradable packaging, coatings, and biomedical fields. Further research should focus on the mechanical properties, polymerization behavior, and large-scale production feasibility to advance its practical applications.

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