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ORIGINAL ARTICLE

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Preparation and Characterization of Renewable Bio-Polyol from the Edible Seed Oil

J.L. Jebisha [1], S. Begila David [2]

[1] *Research scholar (Reg no:21213162032019), Department of Chemistry and research Centre, Scott Christian college (autonomous), Nagercoil-629 003, Tamil Nadu, India.*
(Affiliated to Manonmaniam Sunderanar University, Tirunelveli-627 012, Tamil Nadu, India)

[2] *Research supervisor & Associate professor, Department of Chemistry & research Centre, Scott Christian college (autonomous), Nagercoil-629 003, Tamil Nadu, India.*
(Affiliated to Manonmaniam Sunderanar University, Tirunelveli-627 012, Tamil Nadu, India)

*E-Mail: ***begilarobin@gmail.com***

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Polyol is an organic compound containing multiple hydroxyl groups. This study looked at the possibility of using an edible oil extract from *Salvia hispanica* seeds as a sustainable source for polyols and, eventually, biodiesel or polyurethane. For this, a combination of hydrogen peroxide and acetic acid was used to create new polyol from the aforementioned oil in one-step synthesis. Standard techniques such as physicochemical analysis, phytochemical and basic radical identification, FTIR and NMR were used to characterize the polyol derivative that was extracted from the oil. Antimicrobial activity of both oil and polyol were tested against certain bacteria and fungi. Spectral analysis demonstrated the formation of polyol and this result indicated the possible of using *Salvia hispanica* polyol as a raw material for the preparation of bio-polymers.

Key words: Salvia hispanica oil, polyol, FTIR, NMR, antimicrobial, bio-polymers

The chemical industry has been prompted to explore for new sources of renewable resources as raw materials by the strong demand for products with petrochemical origins, as well as their unfavorable environmental effects and the growing scarcity of these non-renewable resources. Due to nature's enormous synthetic potential and many green chemistry principles, these raw materials have significantly aided the plastics industry's sustainable development (Eissen *et al.,* 2002; Tian *et al.,* 2012). Due to their availability, low toxicity, biodegradability, natural fluidity, and affordability, vegetable oils are among the most popular alternatives (Biermann *et al.,* 2000; Güner *et al.,* 2006; de Espinosa, *et al.,* 2011). To create polyols, a number of vegetable oil molecules must undergo a chemical transformation. These bio-polyols are then used, among other things, to create polyurethanes (Zhang *et al.,* 2015), polyesters (Chaudhari. *et al.,* 2015), and epoxy (Fernandes *et al.,* 2017). Vegetable oil polyols are fascinating substances with applications as corrosion inhibitors (Kosari *et al.,* 2014; Yoo *et al.,* 2012), tensioactive agents or organogelators (Stan *et al.,* 2008), and useful monomers for the production of diverse macromolecular compounds (Ji *et al.,* 2015). Polyols have been created using natural oils like castor (Ugarte *et al.,* 2014), soybean (Ji *et al.,* 2015), camelina (Balanuca *et al.,* 2015), palm kernel (Septevani *et al.,* 2015), jatropha (Saalah *et al.,* 2015), and rapeseed (Zieleniewska *et al.,* 2015; Kuranska *et al.,* 2015).

An herbaceous plant called chia has opposing, serrated leaves that range in size from 1.5 to 3 inches long and 1 to 2 inches wide. Oval in shape, seeds are roughly 2 mm (0.08 inches) in length and 1 mm (0.04 inches) in width. The lustrous seeds contain darker irregular marks or specks on them and their coat can range in hue from cream to charcoal grey. An annual herb called chia (*Salvia hispanica L*.) blooms in the summer. It is about a meter tall and has opposite, petiolate, serrated leaves that range in length from 4 to 8 cm a width of 3 to 5 cm (Ayerza *et al.,* 2010).

Therefore, this study concentrates in the obtention of new bio-based polyol from the edible oil extract from the seeds of the *Salvia hispanica* and also to test the phytochemicals and basic radicals present in the oil extract. In addition, we characterize the prepared polyols using various physical properties and spectroscopic methods. The application of the prepared polyol against certain microbes were also tested.

MATERIALS AND METHODS

OIL EXTRACTION:

The extraction of oil 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). In a Soxhlet device, 5g of *Salvia hispanica L.* seeds were extracted with 100 ml of n-hexane as the extraction solvent. Following an 8-hour period, the n-hexane was eliminated at 40˚C and low pressure. After obtained, the *Salvia hispanica L*. oil (SHO) was kept in a refrigerator till additional research was conducted.

SYNTHESIS OF POLYOL:

The synthesis of the polyols was done in accordance with the approach outlined by Monteavaro et al. (2005). 5g (5.6 mmol) of oil 9.30 mL (0.162 mol) of glacial acetic acid in 20 mL of toluene, together with a few drops of sulfuric acid, were combined in a three-necked flask that was fitted with an isobaric funnel, reflux condenser, and mechanical stirrer. At room temperature, the mixture was mechanically agitated until it was completely homogenized. Subsequently, 5.30 mL of a 30% H_2O_2, solution was added gradually while maintaining the temperature. Following the addition of H_2O_2, the mixture was heated for 12 hours to 60˚C. After bringing the reaction mixture down to room temperature, surplus peroxide was removed by stirring it for 20 minutes while adding a 10% (w/v) sodium bisulfide solution. Following that, the mixture was mixed with 50 mL of ethyl ether, and the organic phase was repeatedly washed to a pH of neutral using a 10% (w/v) sodium carbonate solution. In order to extract *Salvia hispanica* L. (SHP), the organic phase was lastly dried over sodium sulphate and concentrated under vacuum to remove the ethyl ether.

CHARACTERISATION OF OIL AND POLYOL:

PHYSICO CHEMICAL ANALYSIS:

The acid value of SHP was determined by the volumetric titration method according to ASTM D1980-

87. The known quantity of sample is dissolved in neutral butanol-toluene mixture and 3–4 drop of phenolphthalein indicator added, swirling gently and titrated against the 0.1 N alcoholic KOH solution. Equation (1) was used to determine the acid value of SHP.

$$\text{Acid value} = \frac{B \text{ X N X } 56.1}{W} \quad (1)$$

where *B* = Burette reading (ml), *N* = Normality of alcoholic KOH solution, *W* = weight of sample (g).

The hydroxyl value of SHP was determined by acetic anhydride-pyridine method according to ASTM D4274-16. The calculation of hydroxyl value was done by using Equation (2).

$$\text{Hydroxyl value} = \frac{(B - S) \times N \times 56.1}{W} \quad (2)$$

where *B* = Burette reading for blank (ml), *S* = Burette reading for sample (ml), *N* = Normality of alcoholic KOH solution, *W* = weight of sample (g).

PHYTOCHEMICAL AND BASIC RADICAL IDENTIFICATION:

Phytochemical screening and basic radical identification were performed to asses the qualitative chemical composition of different samples of crude extracts using commonly employed precipitation and colouration reactions to identify the major and secondary metabolites.

FTIR ANALYSIS:

The chemical structure of the SHO and SHP were identified by FTIR on a Bruker ATR spectrophotometer. The spectra were observed in the 600 – 4000 cm^{-1} wavelength range.

^{1}H NMR SPECTROSCOPY:

The supportive confirmation of chemical structure was given by ^{1}H nuclear magnetic resonance (NMR)spectroscopy. ^{1}H spectra of the product were analyzed using Bruker DPX 400 MHz spectrophotometer with $CDCl_3$ as solvent.

ANTIMICROBIAL ACTIVITY:

Antimicrobial activity was examined using “The Kirby-Bauer Method” against the number of pathogens including both gram-positive and gram-negative bacteria and also certain fungi. The zone of inhibition of both SHO and SHP were examined against certain pathogens.

RESULTS AND DISCUSSION

PHYSICO CHEMICAL ANALYSIS:

The acid number is expressed as the number of milligrams of KOH required to neutralize the acidity of sample. The OH number is the amount of available reactive hydroxyl groups on polyol molecules. The viscosity was measured using Brookfield viscometer and the density of the polyol were also analysed. The SHP is well dissolved in Chloroform**.** The results of the certain physico chemical analysis were mentioned in the Table 1.

PHYTOCHEMICAL SCREENING:

The preliminary phytochemical study reveals the presence of glycosides, reducing sugars, phenolic compounds and saponins (Table 2) in the SHO. The color change in the extract were also listed in the table.

BASIC RADICAL IDENTIFICATION:

Basic radical test shows the presence of bismuth, barium calcium and magnesium in the SHO were listed in the Table 3.

FTIR:

The successful conversion of SHO to SHP was confirmed qualitatively by FT-IR spectroscopy. Fig. 1 and Fig. 2 shows the FTIR spectra of SHO and SHP respectively. The SHP spectra shows a broad band at 3562 cm^{-1} which were assigned to the presence of a hydroxyl (–OH) stretching vibration. A strong band at 3181 cm^{-1} was attributed to the presence of aromatic -CH-stretching and bands at 1644 cm^{-1} and 1400 cm^{-1} were assigned to the presence of amide band from protein carbonyl stretches and C-O-H bending vibrations. The appearance of new peak at 3562 cm^{-1} in SHP spectra confirms the formation of polyol.

1-H NMR spectra:

Fig. 3 and Fig. 4 gives the 1-H NMR spectra of the SHO and SHP respectively. The methylene protons of the aliphatic chains may be responsible for the signal seen as a triplet between 1.63 to 1.67 ppm (3H) in Fig. 4. The proton of the hydroxyl group is represented by the sharp singlet peak at 2.731 ppm (6H); the electronegative influence of the oxygen atom is responsible for the absorption shift towards the downfield. The multiplet signal at 3.8 ppm (2H) is indicative of the protons next to the acid’s carbonyl

group. The peak shift downwards as a result of the carbonyl group's electronegative action, which de-shield the area (Dai *et al.,* 2005). The signals corresponding to the toluene structure were found at 7.5-7.6ppm as multiplet (Fig. 4). This evidence confirmed that the elimination of toluene was ineffective. Furthermore, the olefinic hydrogen (-CH=CH-) signal that had been present in the range of 5.3 to 5.3 ppm (Fig. 3) in SHO vanished, indicating that the SHP structures were essentially unsaturated. Lastly, the hydroxylation reaction was verified by the lack of signals in the range of 2.8 to 3.3ppm in relation to the epoxide groups (-CH(O)CH-).

Table 1: Physico chemical analysis of polyol

Parameters	**Chia based polyol**
Acid number (mg KOH/g)	5.386
OH number (mg KOH/g)	166.54
Density (g/cm^3)	0.868
Viscosity (cps)	464
Solubility	Chloroform

Table 2: Phytochemical screening of SHO

EXPERIMENT	**OBSERVATION**	**INFERENCE**
NaOH test Extract + 2ml of NaOH	No blue green color obtained	Absence of anthocyanin
Bromine water test: Extract + bromine water	Pale yellow color obtained	**Presence of glycosides**
1ml of + lead acetate solution	No precipitate obtained	Absence of phenol
5 ml of extract + 2ml of $CHCl_3$ + 3 ml of conc. H_2SO_4	No reddish-brown precipitate obtained	Absence of terpenoids
LEAD ACETATE TEST: Extract + 1ml of lead acetate	No red precipitate	Absence of tannins
Extract + $CHCl_3$ + conc. H_2SO_4	No purple color obtained	Absence of steroids
Extract + Molisch's reagent	Purple color is obtained	**Presence of reducing sugars**
Extract + 2N HCl and remove the aqueous layer and add Mayer's reagent	No white precipitate obtained	Absence of alkaloids
Alcohol + extract + ferric chloride	Intense color	**Presence of phenolic compounds**
Extract + water + shake well	Foamy lather is obtained	**Presence of saponins**
Alcohol + extract + Mg ribbon + Conc. HCl	No color change	Absence of flavonoids
Alcohol + Conc. HNO_3 + NH_3	No reddish orange color is obtained	Absence of xanthoproteins

Table 3: Basic radicals' identification

EXPERIMENT	**OBSERVATIONS**	**INFERENCE**
Extract + KI	No golden spangles	Absence of lead
Extract + NH_4OH	white precipitate	**Presence of bismuth**
Extract + cupron reagent + NaOH	No green color obtained	Absence of copper
Extract + potassium ferrocyanide	No white precipitate obtained	Absence of zinc
Extract + dil. HCl + water + H2S	No yellow precipitate	Absence of cadmium
Extract + potassium thiocyanate	No red or blue color	Absence of ferric and cobalt
Extract + dil. HCl + aluminon reagent + $(NH_4)_2CO_3$	No bright red precipitate is obtained	Absence of aluminum
Extract + conc. HNO_3 + sodium bismuthate + water	No pink color	Absence of manganese
Extract + dimethyl glyoxime + NH_4OH	No scarlet red precipitate	Absence of nickel
Extract + potassium chromate	Pale yellow precipitate	**Presence of barium**
Extract + NH_4OH + ammonium oxalate	White precipitate	**Presence of calcium**
Extract + Magneson reagent + NaOH	Blue precipitate	**Presence of magnesium**
Extract +NaOH + Nesseler's reagent	No reddish-brown precipitate	Absence of ammonium

Table 4: Shows the zone of inhibition against certain microbes

Bacteria	Zone of inhibition		
	SHO	**SHP**	**Control (Amikacin)**
Proteus mirabilis	12 mm	11 mm	18 mm
Salmonella typi	13 mm	14 mm	19 mm
Fungi	**Zone of inhibition**		
	SHO	**SHP**	**Control (nystatin)**
Aspergillus niger	10 mm	8 mm	15 mm
C. tropicalis	15 mm	14 mm	13 mm

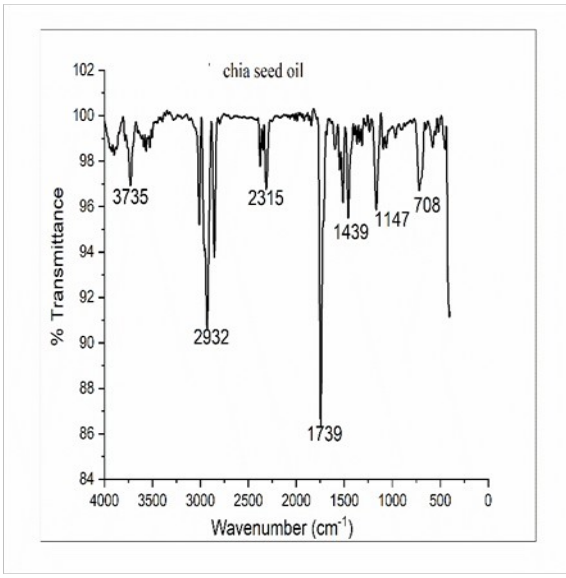

Figure 1. FTIR spectra of SHO

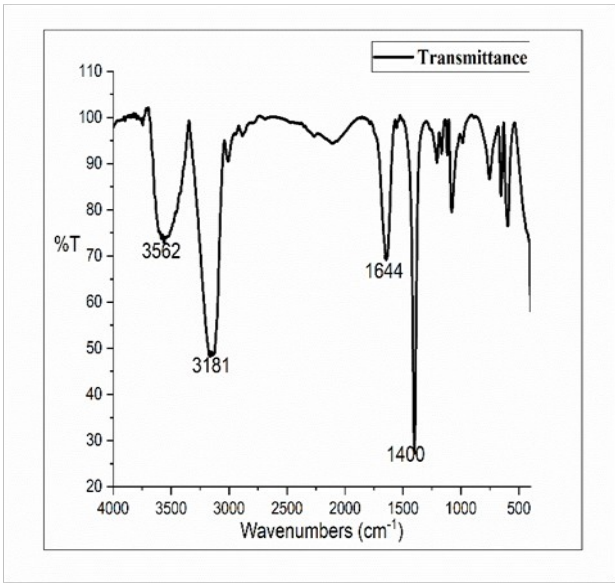

Figure 2. FTIR spectra of SHP

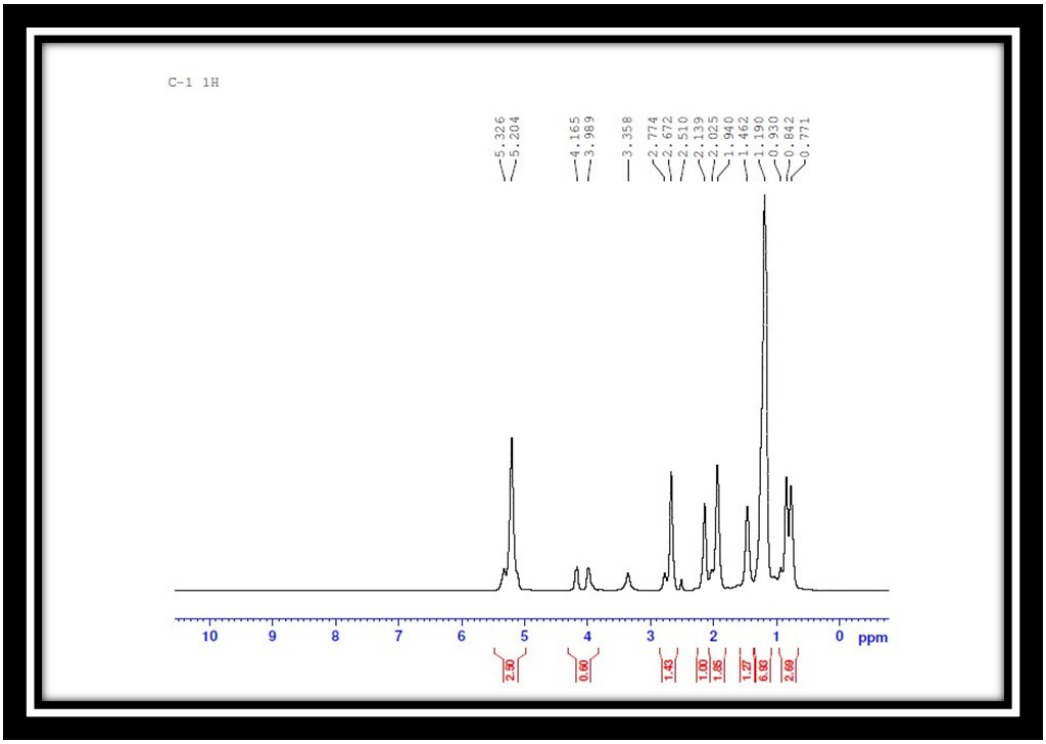

Figure 3. 1-H NMR spectra of SHO

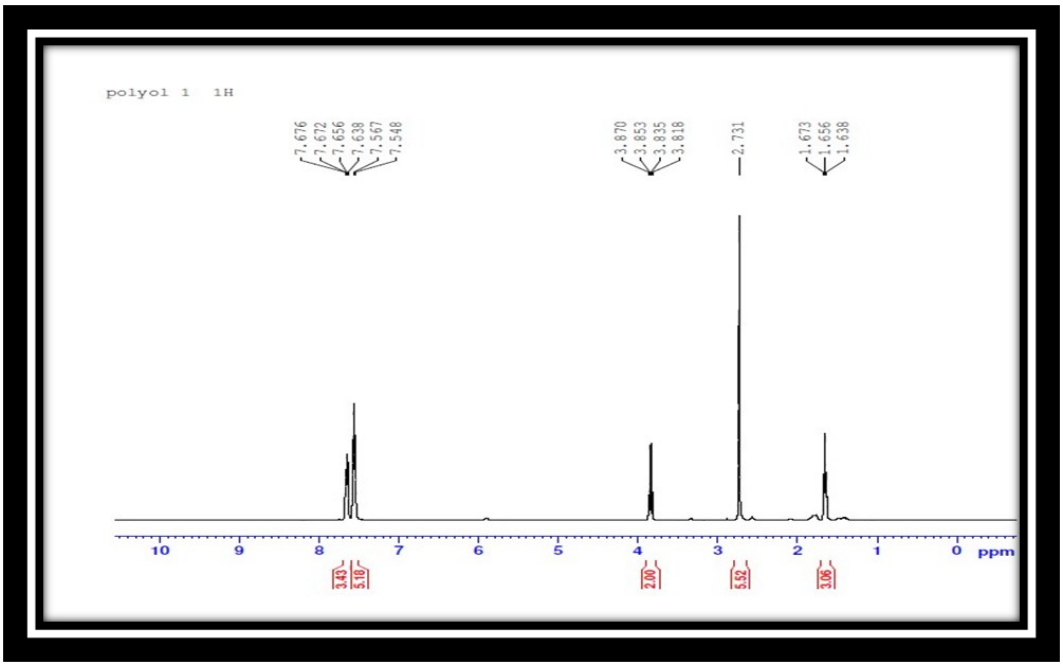

Figure 4. 1-H NMR spectra of SHP

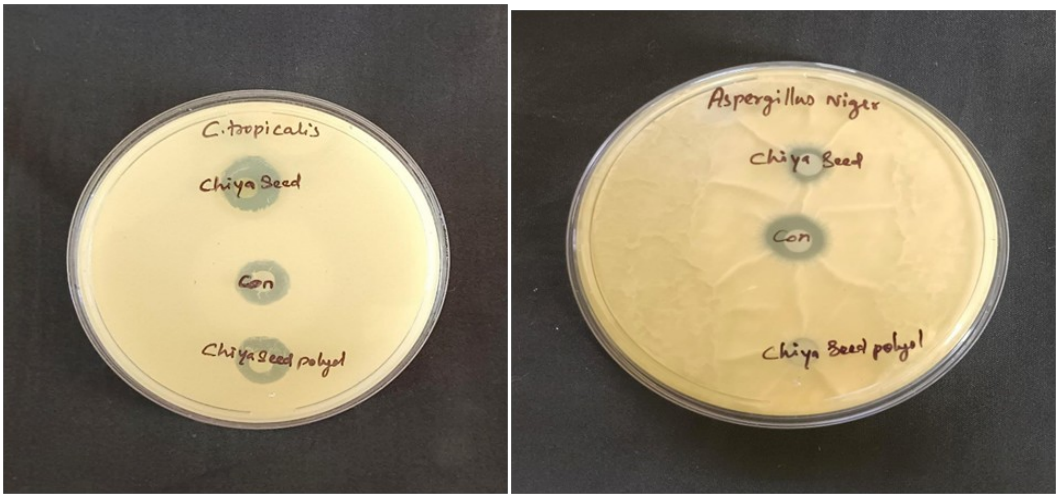

Figure 5. Zone of Inhibition against fungi

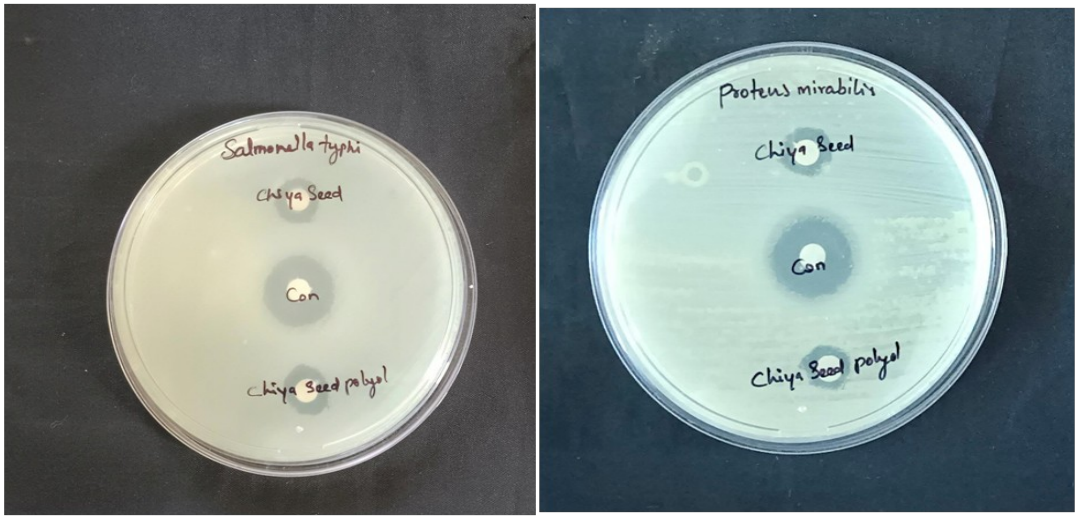

Figure 6. Zone of Inhibition against bacteria

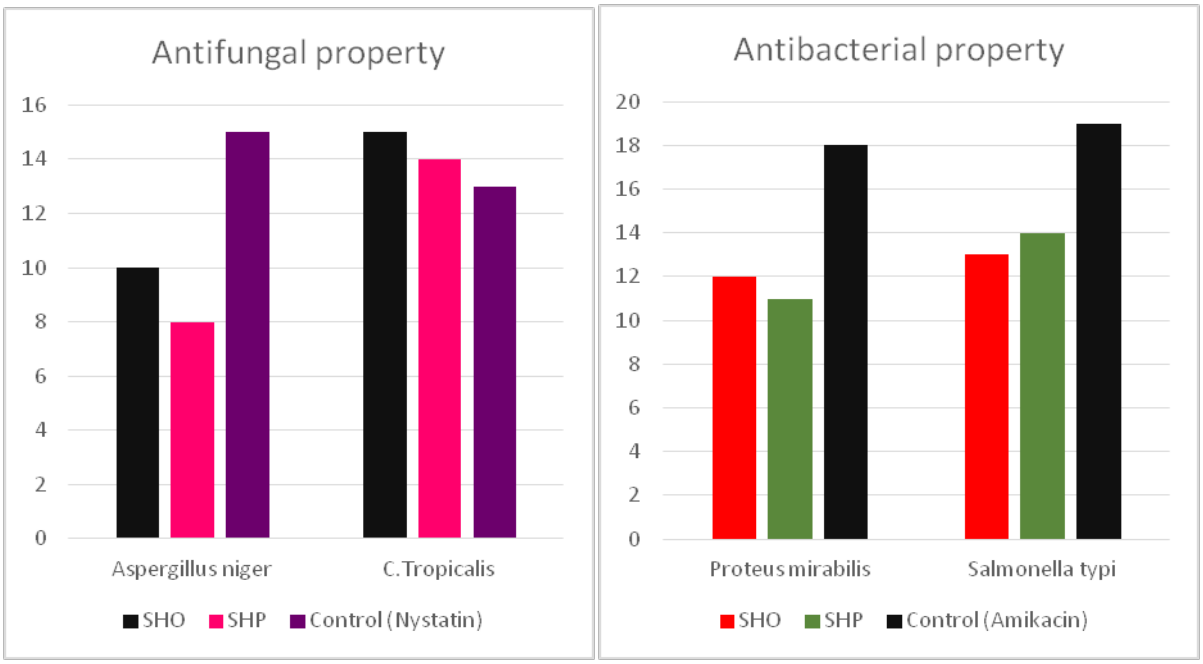

Figure 7. Comparative study

APPLICATION:

ANTIMICROBIAL ACTIVITY:

Two bacterial cultures (Fig. 6) including *Proteus mirabilis* (gram positive), *Salmonella typi* (gram negative) and two fungal cultures (Fig. 5) including *Aspergillus niger* and *C. tropicalis* were used to check the antimicrobial potential of both SHO and SHP. Amikacin was used as a control against bacterial cultures while Nystatin was used as a control against fungal cultures. The zone of inhibition against both bacteria and fungi were listed in the Table 4. The bar graph shows the comparative study of SHO and SHP against certain microbes (Fig. 7).

CONCLUSION

We can infer from the results above that SHP was successfully prepared from the hexane extract of *Salvia Hispanica* and it was characterized by using FTIR and ^{1}H NMR spectroscopy. The physico-chemical analysis of the SHP and phytochemical screening and basic radical identification of SHO were examined. The antimicrobial potential of the prepared polyol and the oil were tested against certain bacteria and fungi. Thus, the prepared bio-polyol can be used as an alternative for organic polyols which can be effectively use for the preparation of certain bio- polymers.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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