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RESEARCH ARTICLE

MODIFIED TANNIN EXTRACTED FROM *THUJA ORIENTALIS* AS ENVIRONMENTAL-FRIENDLY ANTIBACTERIAL AND ANTIOXIDANT

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Abstract

The present investigation represents the efficiency of tannins, a flavonoid-type normal poly phenolic aggregate, its synergistic impact with business type. The antimicrobial movement of has been generally abused for avoidance of nourishment – borne pathogens e.g: *Escherichia coli* being the significant reason for the runs particularly in children, in view of bacitracin action and the significance of home grown medications, thus this examination was intended to assess the impact of plant remove from *Thujaorientalis* is a typical elaborate evergreen tree. Assume a significant job in securing against harm by responsive oxygen species. It expected to examine the impacts cell reinforcement supplements in the avoidance keeping up human wellbeing. Scrap tire rubber is among a greater waste polymers. It doesn't disintegrate effectively inferable from its cross connected structure. Alteration of scrap tires powder by the joining Tannin with squander rubber was portrayed by FT-IR, SEM, TGA, XRD and DSC estimations.

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Introduction:-

The world devours 4.5 million tons of tires, notwithstanding billions of feels burnt out on various sorts are to be discarded in the USA. This enormous measure of scrap tires is a major worry as natural contamination issue (Sultan and Jabrail, 2019). While it could be used as an elective wellspring of vitality if this piece is changed into valuable material. This part comprises of around 60-65% rubber (Jastrzębska, 2019). Accordingly, disconnection of the rubber from different added substances should be an important method to acquire a valuable material which could be prepared and reused in a few mechanical strategies. The rubber altered folio process uses an all the more finely ground morsel rubber (Cheng, Chen, Huang, Li, and Guo, 2012; Sultan and Jabrail, 2019). Strong waste administration is one of the major ecological concerns around the world, applications where tires can be used have demonstrated to be successful in ensuring the earth and rationing common assets. As of late with the expansion in the interest for framework and possible establishment plan in not pertinent because of poor bearing limit of ground has begun to take another shape (Hambirao and Rakaraddi, 2014). That implies that the enthusiasm for reusing tires to modify them is diminishing. This is most likely because of the troublesome and requesting procedure of recovering and re-vulcanizing the rubber that is utilized in new tires (Bressi, Fiorentini, Huang, and Losa, 2019; Li et al., 2019). The use of the reused rubber from the End of Life Tires. The point of this study was to change the dry strategy for blending morsel rubber modifier with Tannins. Tannins are polymeric phenolic mixes with various hydroxyl gatherings and very differing in synthetic structure Hydrolysis of some of tannins yields (Altemimi, Lakhssassi, Baharlouei, Watson, and Lightfoot, 2017; Ishak and Elgailani, 2016). Plant inferred bioactive substances are acceptable wellspring of meds that assume a critical job for human wellbeing and furthermore utilized against

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various sorts of microbial disease (Dash, Mishra, and Dash, 2014a, 2014b; Sruthi and Indira, 2016). A plant of the family Cupressaceous, *Thujaorientalis* (normally morpankhi, family Cupressaceous) is an evergreen and monoecious tree or bush has been utilized in the diverse movement that is, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic is utilized for the treatment of bronchial catarrh, stiffness, psoriasis, amenorrhea, cystitis, uterine carcinomas, and headache, and as an abortifacient, preventative, antidiarrheal, and hepatic defensive medication in people medicine (Akkol et al., 2015; Jain and Sharma, 2017; Srivastava, Kumar, Singh, and Singh, 2012).

Experimental

Chemicals

All chemicals and reagents used in this study were of analytical grade and obtained from Merck Company, Germany

Characterization

Infrared spectroscopy analysis (FT-IR)

Polybutadiene-g-Tannin powder was mixed with KBr powder pressed into a round tablet and analyzed using an infrared spectrometer .

Scanning Electron Microscopy

At the point when an electron pillar encroaches the example surface, low-vitality electrons as optional electron can be identified inside the couple of angstroms of the example surface by a scintillator finder. SEM assumes a significant job to decide surface morphology of polymers (Lee and Lee, 2010).

Thermogravimetric analysis

Thermogravimetric examination (TGA) was utilized to assess the progressions of substance structure of GTR and Tannin-g-polybutadiene. TGA was acted in a nitrogen air, Test temperature went from 30 to 800 C at a warming pace of 10 C/min (Hussein Ali Shnawa, 2017).

Differential scanning calorimetric analysis (DSC)

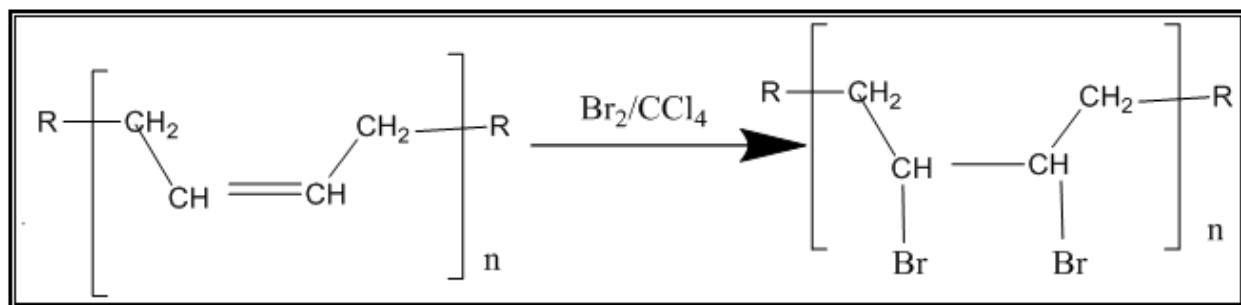
Differential checking calorimetric (DSC) tests were performed utilizing a DSC model estimations were additionally completed for additional research the adjustment impacts of tannin-g-polybutadiene. The adjustments in warm progress conduct (glass change temperature) of the composites were noted by DSC-60Mettler Toledo, Switzerland (Suhailath et al., 2017).

Materials And Experimental Procedures:-

The ground Scrap tires were gathered from Basra scrap tire dump site, Iraq. The tires were washed with water to evacuate earth and were consequently air dried. The cleaned sides of the tire liberated from steel breeds were cut into segments with the guide of a hacksaw and later into little pieces utilizing sharp blades the a normal molecule size 0.2 - 0.4 mm was set up from squander tires (F. Aisien, Amenaghawon, and Adeboyejo, 2013; F. A. Aisien, Amenaghawon, Adeboyejo, and Eng, 2013) .

Halogenation of ground tire rubber

Ground tire rubber after treatment (4.0 g) was added to 25 ml of Bromine in carbon tetra chloride of, the blend was mixed for 2 hours at 25C. The acquired the halogenated ground tire rubber. It was washed by ethanol and dried at 100 C° for 12 hours (Murray et al., 2012; Scagliusi, Cardoso, Pozenato, and Lugão).



Collection and identification of plant.

The fresh plants of *Thujaorientalis*Linn were collected in the months of April from Bashar, Iraq .The plant material was dried under shade at room temperature for about 10 days. The dried plant samples were powdered by mechanical grinder.

Isolation oil from *Thujaorientalis*Linn

Powder (34 g) was filled in the thimble and extracted successively with n- hexane as solvents in soxhlet extractor for 24 h. The extracts were concentrated to dryness using rotary evaporator(Sharma & Sharma, 2016).

Isolation tannin from *Thujaorientalis*Linn

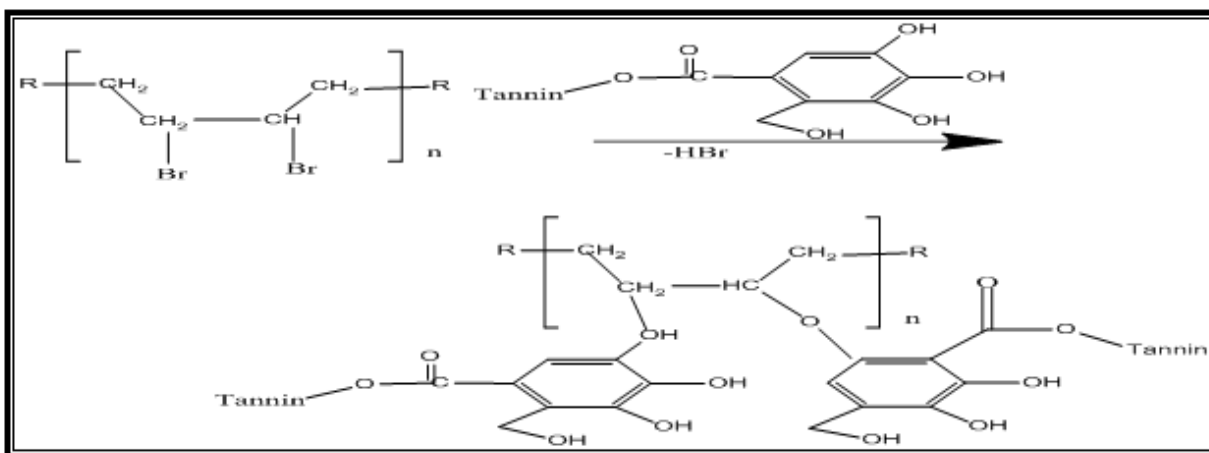
Take (50g) of leave isolated oil of *Thujaorientalis*Linn with 70% ethanol (500 mL). The homogenates were continuously stirred for 12 h at room temperature and filtered over Whitman 1 filter paper, the pH values of each extract have been recorded(Regmi, 2018).

Tannins Test

2ml of the extract was treated with two drops of 1% FeCl₃. The formation of a green precipitate on dilution indicates the presence of Tannins (Musa).

Modification of halogenated ground tire rubber with Tannin

(6g) of halogenated ground tire rubber were dissolved in 25 ml of DMF and 3g from tannin in 25ml DMF were mixed together at 110c° with stirrer . The reaction continuous for one hour after that it was stopped , acidification with 10% v/v H₃PO₄. The product filtered and precipitated in cold ethanol. The modified polymer was purified using THF(Hussein A Shnawa, Khaleel, & Muhamed, 2015).



Antibacterial Susceptibility

The antibacterial activity of modified compound have been tested against tow cultures of pathogenic bacteria (*Escherichia coli*, *Staphylococcus aurous*) following the method reported by using drop diffusionmethod. The bacterial culture was grown on nutrient agar and incubated at 37°C for 24h. Then was applied followed by incubation for additional 24h. The antibacterial activity was estimated by measuring the diameter of inhibition zone(Jaiswal, Kumar, Mishra, & Kasula, 2011).

Determiation of antioxidantactivity

The antioxidant activity of the prevention agent action of the Tannin altered was assessed used β -carotene-linoleate model framework as showed by with some alteration. 0.1 mg of β -carotene in 0.2 mL of chloroform, 10 mg of linoleic corrosive and 100 mg of Tween-20 (polyoxyethylenesorbitanmonopalmitate) were blended. Chloroform was evacuated at 40 °C under vacuum and the subsequent blend was weakened with 10 mL of water and was blended well. To this emulsion, 20 mL of oxygenated water was included. Four milliliter aliquots of the emulsion were pipetted into various test tubes containing 0.2 mL of Tannin-g-polybutadiene and BHT 50mg/l in ethanol BHT was utilized for relative purposes. A control containing 0.2 mL of ethanol and 4 mL of the above emulsion was readied. The cylinders were put at 50 °C in a water shower and the absorbance at 470 nm was taken at zero time (t=0). Estimation of absorbance was proceeded till the shade of β -carotene vanished in the control tubes (t=60 min) at an

interim of 15 min. A blend arranged as referenced above without β -carotene filled in as clear. All conclusions were done in triplicate. The cancer prevention agent movement (AA) of the concentrates was assessed regarding blanching of the β -carotene utilizing the accompanying equation,

$$AA = 100[1 - (A_o - A_t) / (A_o^o - A_t^o)]$$

where A_o^o and A_t^o are the absorbance esteems estimated at zero time of the hatching for test and control, separately. A_t and A_t^o are the absorbance estimated in the test and control, separately, after hatching for 60 min. The outcomes were communicated in % premise of forestalling fading of β -carotene(Djuric and Saso; Reksamunandar, Edikresnha, Munir, and Damayanti, 2017).

Results And Discussion:-

FT-IR analysis

The poly phenolic tannin compound have many characteristics bands at certain frequencies. Its FT-IR spectrum is shown in (Fig. 1) broad peak at 3397cm^{-1} is attributed to polymeric O-H group, and the wideness of the 1708 cm^{-1} band can be related to the presence of conjugated carbonyl groups.the frequency at 2930 cm^{-1} corresponds to C-H stretching frequency,. A notable band at 1030 cm^{-1} can be assigned to C-O stretching. The presence of the functional group C-O-C in tannin is confirmed from the band at 1207 cm^{-1} At 759 cm^{-1} shows the result distortion vibration of C=C in benzene rings. The absorption band at 871 cm^{-1} , corresponds to the characteristic absorption of β -D- glucose unit.

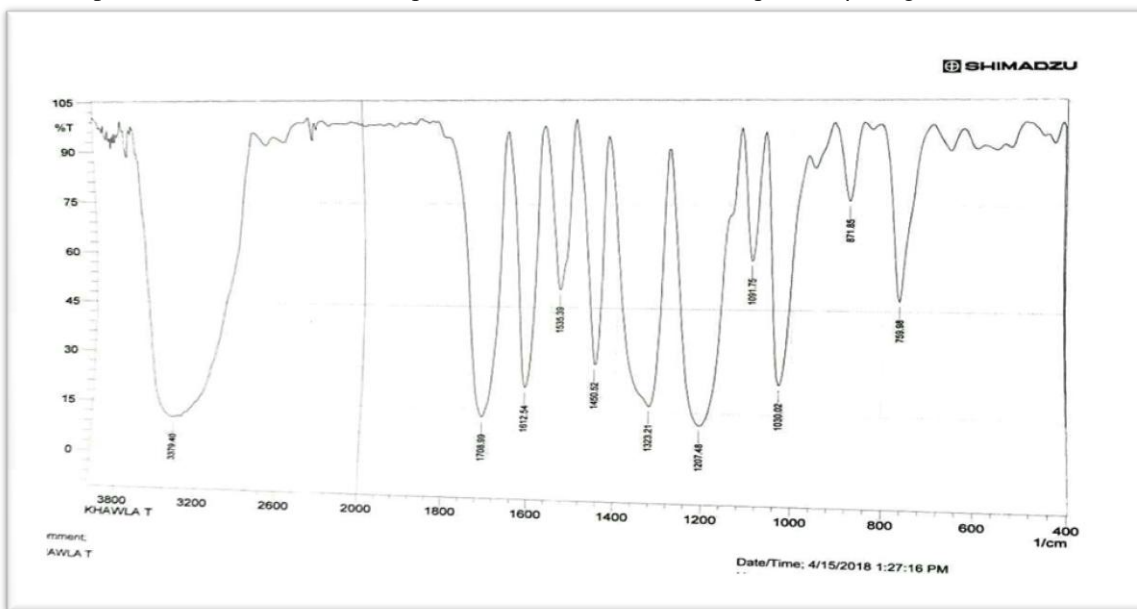


Figure (1) FT-IR spectra of Tannin

Infrared spectrum of Tannin-grafted-polybutadiene

Grafting Tannin with polybutadiene, (Fig. 2), exhibits the showed the characteristic peaks at 3479 cm^{-1} assigned for O-H stretching and new intense bands in the region $2920\text{-}2854\text{ cm}^{-1}$ attributed to the C-H stretching vibration of the methylene group, which confirms the occurrence of hydroxyl methylation for phenolic tannin. The C-O stretching vibration of the hydroxyl groups showed very strong band in 1030 cm^{-1} .

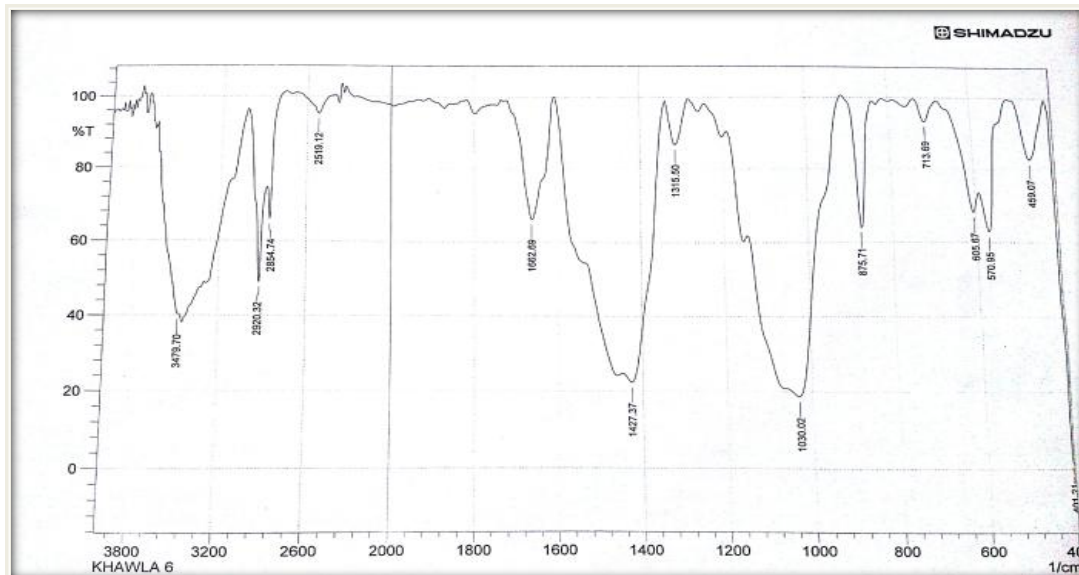


Figure (2) FT-IR spectra of Tannin-g-polybutadiene

Phytochemical screening

Phytochemical screenings were performed using standard procedures.

FeCl ₃ Test	+	+
5% FeCl ₃ solution	+	+
Reaction with lead acetate	+	+

Thermogravimetric analysis

The thermal stability of all Tannin and Tannin-g-polybutadiene definitions was examined utilizing thermogravimetry (TG) procedure.

From thermogram of the tannin debasement, three particular mass misfortune pinnacles can be found in (Figure 3), seven days top focused at 117 C° where, warm improving, fundamental oxidation steps and end of unpredictable parts. The subsequent pinnacle is more honed and increasingly articulated and it is found at 301 C° Third corruption of tannin happens after at 450 C° with comment top at 580 C° is seen with of carbon buildup right now. The Tannin - g-butadiene polymer, three phases of decay are appeared in (Figure 4). The principal arrange at 90 C ° identified with the loss of water atoms. The second stage at 282°C can be identified with the misfortune from the cleavage of the methylene gathering .The third stage is misfortune at 500 Co relates to the depolymerization of tannin and polybutadiene rubber.

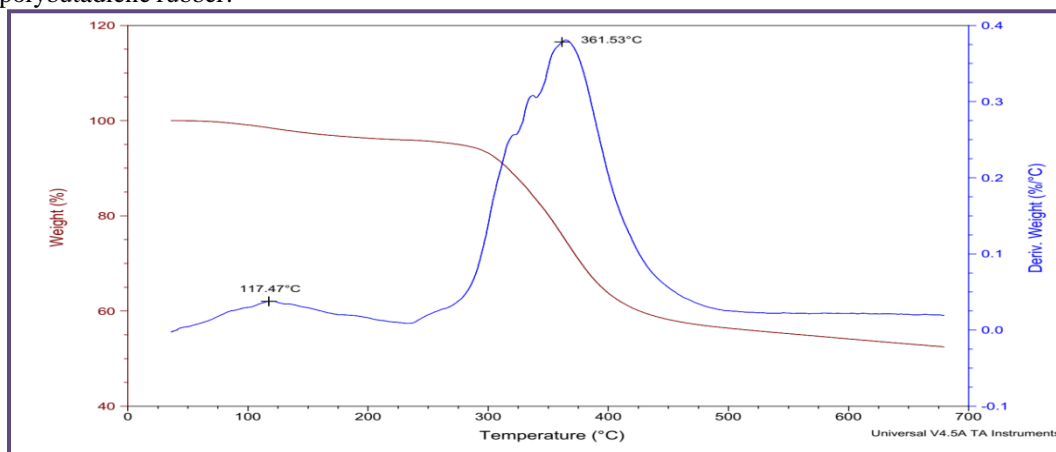


Figure (3) Thermogravimetric diagram of Tannin

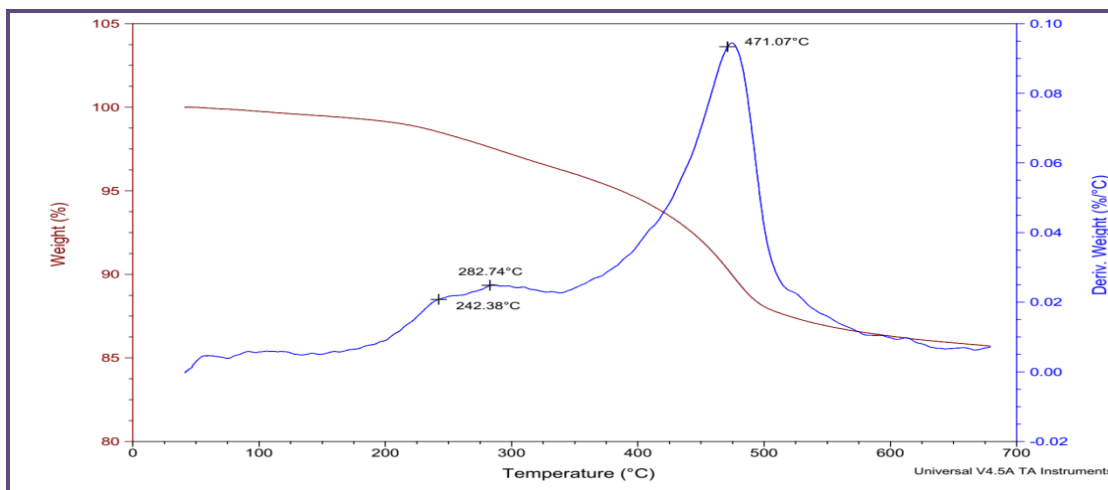


Figure (4) Thermogravimetric diagram of Tannin-g-polybutadiene

The DSC curve of Tannin-g-polybutadiene thermogram (Fig.5) which showed small two endothermic peaks, the first one is at 209 C° the glass transition temperature is appeared, at T g region, the polymeric chain becomes more soft and rubber like Glass transition temperatures, The second endothermic peak 232.6 C° corresponded to the chemical bonds decomposition.

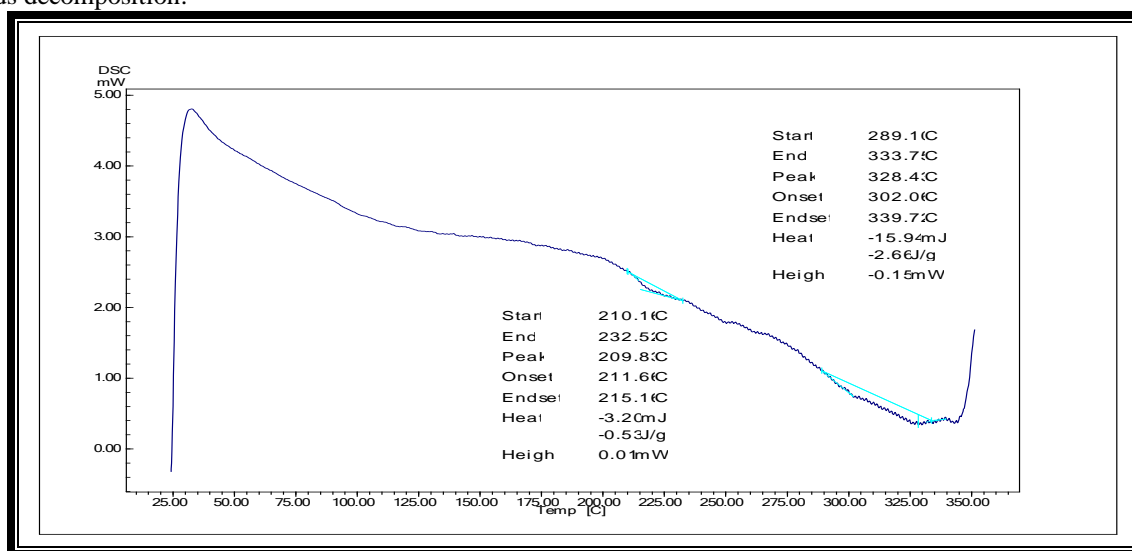
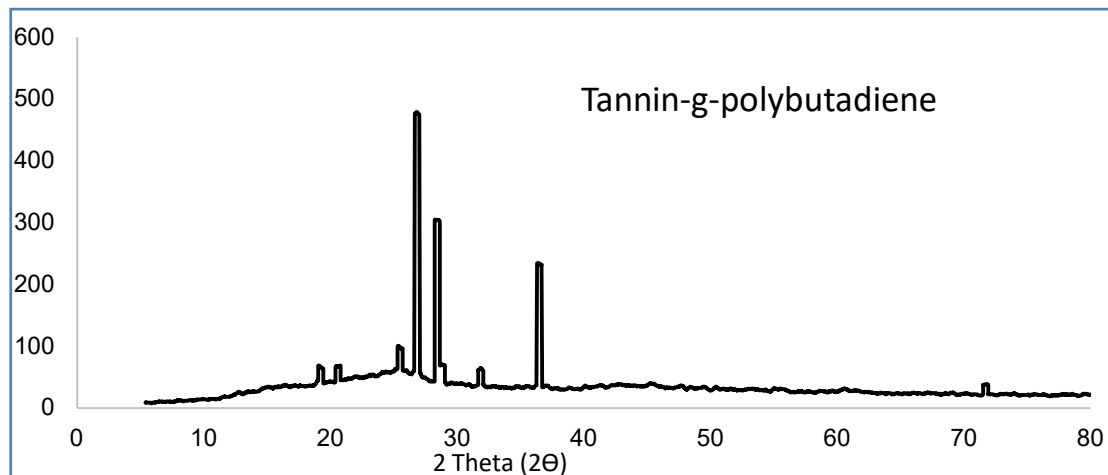


Fig. (5) DSC thermogram curve of Tannin-g-polybutadiene

X-ray Diffractometry:

Diffraction is a physical wonder that comprises in electromagnetic waves evading snags if the size of the hindrances looks at to the wavelength. This marvel can be applied to the investigation of materials as the iota plans are put at equivalent separations to X beam lengths. X beams are electromagnetic waves like light, yet whose wavelength is a lot shorter ($= 0, 2 - 200 \text{ \AA}$) (Elena and Lucia, 2012). It was seen that the X-beam diffractogram Figure (6) of Tannin – g-polybutadiene reflections at 2θ of 51° . It was seen that the X-beam diffractogram of polymer sub-atomic chains has gotten more fragile and the level of normality is diminished.



Fig(6):- X-ray Diffractometry Tannin-g- polybutadiene.

Morphology analysis

Figure 7 shows the SEM images the surface morphology of modified samples for microstructural analysis, studied by SEM, is given in Figure 7. It is clear from Tannin-g-polybutadiene show an These images showed different sizes and shape spherical were distributed. The surface of the sample showed bead-like structures. The size of values minimum and maximum of 39-197 nm.

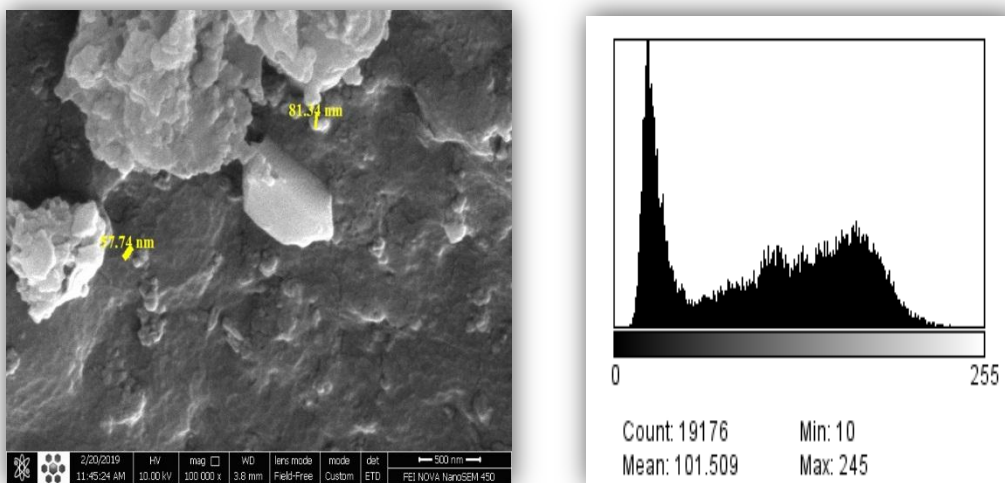


Figure (7):- Size of (Tannin-g-pBut) using J -Image program.

Antibacterial Susceptibility of the modifying polymer

Microorganisms are living organisms, for example, microscopic organisms, growths, and parasites, which are the basic sources of contaminations, Infectious illnesses result from pathogenic microorganisms and murder a bigger number of individuals than some other single cause(Huang et al., 2016). Antimicrobials assume a significant job in creature and human social insurance. They are used for treatment and anticipation of irresistible infections in domesticated animals and to shield general wellbeing from nourishment borne maladies An unavoidable reaction of the utilization of antimicrobials is the rise and spread of safe microorganisms (Burow, Simoneit, Tenhagen, and Käsbohrer, 2014; Gaamoune, Harzallah, Kada, and Dahamna, 2014)

Antibacterial action of all the adjusted polymer from ground tire rubber was completed against tow bacterial strains. The positive, Staphylococcus aureus, and the negative, Escherichia coli. Zones of hindrance were estimated Figure (8).

Compounds & cod	The diameter of the inhibition zone in millimeters	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
Tannin-g-polybutadiene	57	60



Figure (8) Tannin-g-polybutadiene

Activity, Antibacterial activity of *Staphylococcus aureus* & *Escherichia coli*.

Determination of antioxidant

What guided us to try β -carotene–linoleic bleaching inhibition assay was the finding that using β -carotene–linoleic bleaching inhibition assay to test if the examined compounds may prevent/inhibit lipid peroxidation. Briefly, in this system, linoleic acid in an oil–water emulsion phase undergoes thermally induced oxidation, thereby producing free radicals which react with the β -carotene's chromophores (Aminjafari et al., 2016; Gong, Smith, Swanson, & Rubin, 2018). β -carotene experiences quick staining without a cancer prevention agent. This reality happens as a result of the coupled oxidation of β -carotene and linoleic corrosive, which creates free radicals. The linoleic corrosive free radical shaped upon the delamination of a hydrogen particle from one of its gatherings assaults the exceptionally unsaturated β -carotene atoms. Accordingly, β -carotene is oxidized and separated partially; in this way, the framework loses its chromophores and trademark orange shading, which is checked spectrophotometrically (Luis, Gil, Amaral, and Duarte, 2012), resulting in a bleaching effect.

comp	A_i	A_{t105}	A_i^*	A_t^*	AA%
BHT	1.728	1.736	0.599	0.432	95.20
Tannin-g-polybutadiene	0.599	0.576	0.599	0.432	86.22

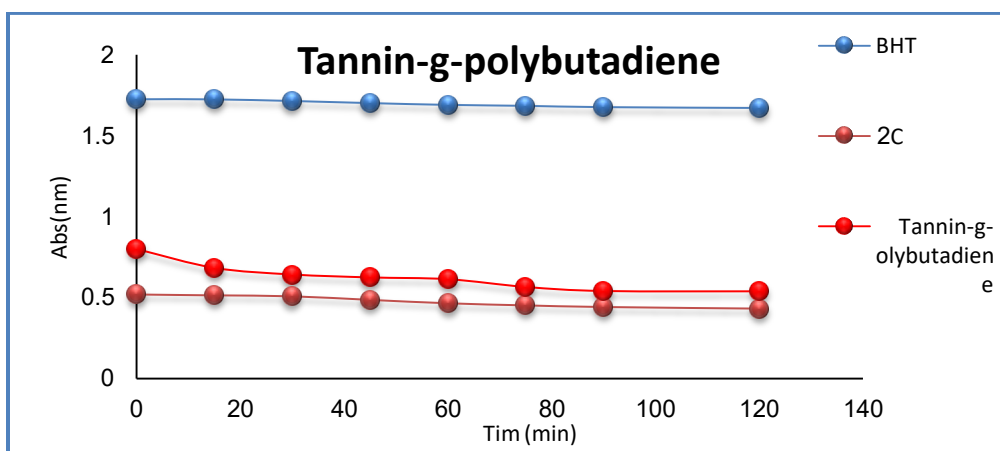


Fig.(9):- The Antioxidant Activity β -carotene Assay the Tannin- g – polybutadiene.

Conclusions:-

Tannin expelled from *Thujaorientalis* is major grafting with significant uniting with polybutadiene from scrap ground tire rubber apparent from this investigation that the invert. That implies that the enthusiasm for reusing tires to reconstruct them is diminishing. This is presumably because of the troublesome and requesting procedure of recovering and re-vulcanizing the rubber that is utilized in new tires. The changed polymer hostile to from Tannin and polybutadiene forestall high movement as against oxidant and Antibacterial action.

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