Antioxidant and bio-activities of leaf extracts from *Eucalyptus globulus*

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Abstract: *Eucalyptus globulus*member of Myrtacaea family, is one of the important medicinal widely used plant.In this study methanolic leaf extract of *Eucalyptus globulus*was studied for the estimation of phytochemical constituents , in-vitro antioxidant and anti-inflammatory activity . Fresh leaves were ground with methanol used for estimations. Phytochemical constituents were estimated by total phenolic content and total Flavonoid content determination. Spectroscopic analysis by UV-VIS, FT-IR and fluorescents analysis was also conducted.*In vitro* anti-inflammatory and antimicrobial activities were also monitored.Phytochemical screening revealed the presence of flavonoids , phenols , carbohydates and saponins. Considerable amount of phenolics (50 mg equivalent par g of dry weight) and flavonoids (23 mg equivalent par g of dry weight) were detected in extract. Extract exhibited considerable DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) scavenging activities, Hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity , Iron reducing power and Superoxide radical scavenging activity. Fluorescent spectroscopy revealed the presence of fluorophores in extracts. A strong anti-inflammatory activity of extract was observed using fluorescent spectroscopy. An appreciable *in vitro* antibacterial activity against gram-negative bacteria like gram-negative bacteria *Escherichia coli*, MTCC 40 was detected.
This study confirmed that methanolic extract *Eucalyptusglobulus*possesses potent antioxidant anti-inflammatory and antibacterial activities possibly important quantity of flavonoid and phenolic content present in it .

Keywords: *Eucalyptusglobules,* flavonoids, reducing power, FT-IR, Fluorescent analysis, *In-vitro* antibacterial activity, anti-inflammatory activity

INTRODUCTION

Aromatic plants are most widely used as source of traditional medicine (Chiang et al., 2003). Literature survey revealed that, despite great progress in synthetic chemistry and western medicine, plants are still backbone of primary healthcare. Worldwide underutilized plants are widely used as herbal medicine in villages (Newman and Cragg, 2016). So detailed investigation of these underutilized plants is need of the hour especially in developing and under developing counties, where primary healthcare strongly relay on traditional drugs. Due to antioxidant properties the consumption of these plants is increasing day by day for development of novel and

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biodegradable effective drugs as alternative to contemporary medicine. Medicinal plants contribution to phytomedicine to the well being of world population, has attracted significant amount of interest from all disciplines. Plant based herbal formulations are healthier, safer and more reliable than synthetic medicines (Tlili et al., 2010). Medicinal plants have been used as important source of therapeutic drug molecules as they poses secondary metabolites which are potential source of drugs. The major bio-active components are secondary metabolites produced by these plants such as alkaloids, flavonoids, phenols, saponins, generally produced by plants as defense mechanism have been implicated as therapeutics drug molecules in medicinal plants (Gao et al. 2012). These components are reported to suppress redox reactions of free radicals in biological system. Herbal agents are mixture plant parts and their formulations are vital with respect to medicinal, nutrient and antioxidant values .

*Eucalyptus globules,*native to Australia, Tasmania and also in Africa and tropical to Southern temperate America,is one of the important medicinal plant widely used (Silva et al, 2003). *Eucalyptus globulus*plant belongs to genus eucalyptusMyrtacaea family. Genus *Eucalyptus* includes more than 700 species which are successfully introduced widely (Ahmad et al., 2005). *Eucalyptus* globulus can be grown in variety of climatic environmental modifications but the best known optimum conditions are evident to be found in countries having warmer climate. *Eucalyptus globulus*plant have known to different names according to their place in which they grow. Most commonly they are known as " Australian fever tree ", " Tasmania Blue gum ", " Southern Blue gum " , and " Stingy bark (Goodger et al., 2016). Trade name of *Eucalyptus globulus* called as " Blue gum". The most readily recognizable characteristics of *Eucalyptus* species are like distinctive flowers and fruits (capsules or gum nuts).Eucalyptus globulus has a fresh mint like smell and a spicy, cooling taste and contains various minerals. *Eucalyptus* essential oil is colourlessand a characteristic taste and odour and typical volatility. Essential oil of *Eucalyptus* is highly flammable and contains compounds that are natural disinfectants and pest repellent. Essential oil of *Eucalyptusglobulus* is composed of a mixture of volatile organic compounds including hydrocarbons , alcohols , aldehydes, ketones , acids , ethers and esters (Su et al., 2006) . It is used as an inhalant because 1,8-cineole is a well known medicinal component that causes a sensation of cold and this is accompanied with a facilitated respiration. Thus , its often inhaled in asthma , pharyngitis and related conditions. The oil of

Eucalyptusglobulus are helpful in relieving symptoms of the common cold for example cough lozenges and inhalants. The antimicrobial and antibacterial potential of Eucalyptus has been harnessed for use in some mouthwash and dental preparations. In proper dental health ,*Eucalyptus* appears to be active fighting bacteria that cause tooth decay and periodontitis(Hayat et al.2015). Despite its key role in traditional medicine, studies on *Eucalyptus globules* leaf extracts and its characterization is still not well known. Thus the main objective of the present work is to investigate the methanolic extract of Eucalyptus globulus leaves for the evaluation of phytochemical constituents by spectroscopic techniques and the valorisation of its antioxidant and antimicrobial potentials.

MATERIAL AND METHODS

Sampling site

Eucalyptusglobulus plant leaves were collected from the surroundings of village SofiPind, Jalandhar , Punjab (Figure.1) . The plant was identified at Departmentof Botany and voucher with number BT101 was deposited in herbarium, of Dept of Biotechnology. All the leaves collected were healthy and disease free for the analyzes envisaged.

Extraction and sample preparation:

Fresh leavesof plant*Eucalyptusglobules*was taken and washed , dried and powdered using grinder. 10 gms of powder was then homogenized in 60ml of 80% Methanol. The crude extract was centrifuged for 10min at 10,000rpm at room temperature. After centrifugation, supernatant was collected. The methanolic plant extract was concentrated using the rotatory evaporator system at 40°C . The resulted extract was collected and was stored at 4C for further use .

Standard solution preparations

Chemicals and instruments

All chemicals viz: Glutathione, Rutin, Vanillic, Methanol, Proline, Tannic acid and Glycine Betainewere of analytical grade and purchased from CDH, Pvt Ltd, India. UV-VIS spectroscopy was conducted using dual spectrophotomerer (Elico, India). FT-IR analysis was done using infrared spectrophotometer (Perkin Elmer Spectrum II, Chicago, USA). The

fluorescence spectrum was measured using Spectrophotometer (FL6500 Perkin Elmer Spectrum II, Chicago, USA).

The eight chemical standard solutions were prepared in different solvents . Glutathione Reduced and Glutathione Oxidized both were prepared in distilled water. Rutinand Vanillic acid both were prepared in methanol. Proline , Tannic acid and Glycine Betaine were prepared in ethanol . Ascorbic acid was prepared in distilled water . These standards were prepared in appropriate solvents with concentration ranges from 5to25 mg per ml and stored at 4°C

Total Phenolic Content

Total Phenolic content of Eucalyptus plant extract was calculated spectrophometrically from Folic- Ciocalteus Method with some modifications (Baba et al.2014) .Extract sample was diluted by 1/10 dilution (1mL of extract in 9 mL of water) before performing the assay. 200µl of different Fractions or concentration of plant extract were taken and there volume were made up to 3mL with distilled water. 0.5mL of 10% FolinCiocalteus Reagent was added to these different fractions and mixed them well .Mixture was allowed to stand for 2min and then 2mL of 20% Sodium Carbonate was added . Allowed the mixtures to stand for 60 minutes in the dark (25°C). Absorbance was taken at 650nm using a UV / visible spectrometer. All tests were carried out in triplicates .Gallic Acid Standard of different concentrations were treated in the same manner to generate a calibration curve . The total phenolic content of extract was calculated from calibration curve and results were expressed as mg of Gallic acid equivalent per g dry weight.

Total phenolic content was calculated from the formulae :TPC = C × DF × V/ M , Where , C = concentration of Gallic acid (calculated from standard curve) . V = volume of the extract . M = weight of tissue , DF = Dilution Factor

Total Flavonoid Content

The Total Flavonoid content of crude extract was determined by Aluminium Chloride colorimetric method (Baba et al.2015) . 50 µL of different concentrations of extract were taken and there volume was made up to 1mL with Methanol.4mL of water was added to it and was mixed well. 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added and mixture was allowed to stand for 5min at room temperature. After incubation 0.3 mL of 10% Aluminium Chloride

($AlCl_3$) solution was added and mixture was allowed to stand for 6mins at room temperature.2mL of 1mol/L Sodium Hydroxide (NaOH) solution was added to the mixture and their volume was made up to 10mL with distilled water . The Mixture was allowed to stand for 15mins and absorbance was measured at 510nm using UV / Visible Spectrometer. Rutin Standard of different concentrations was treated in the same manner as the plant extract generate a calibration curve. All tests were performed in triplicate. Total flavonoid content was calculated from a calibration curve and the results were expressed as mg rutin equivalent per g dry weight. Total Flavonoid content was calculated by the formula:TFC = C × DF × V/ M Where , C = concentration of rutin (calculated from standard curve) . V= volume of the extract. M = weight of the extract.

In-vitro Antioxidant activity of leaf extract of *Eucalyptus globulus*:

DPPH Radical Scavenging Activity:

The antioxidant activity of plant extract was determined by 1,1-diphenly-2-picryl-hydrazyl (DPPH assay) following (Khan et al.2012). DPPH solution was prepared by dissolving 3.2mg in 100ml of 82%methanol. 0.2 ml of different concentrations of plant extract were added to the test tubes followed by the addition of 2.8ml of DPPH Solution. Each fraction of mixture was shaken well and kept at room temperature in dark for 1hour. After incubation colour change in colour was observed and absorbance was taken at 517nm.Mixture of 0.2ml of Methanol and 2.8ml of DPPH solution was taken as control while 2.8ml of 82%methanol and 0.2ml of Methanol was taken as blanks. Ascorbic Acid was taken as positive control and was treated in the same manner as the plant extract . All tests were taken in triplicate.Percentage DPPH assay scavenging activity of plant extract and Ascorbic Acid were calculated by the following formulae:% scavenging activity = (Abs. of control - Abs. of fraction / Abs. of control) x 100 .LogIC50 value was calculated by graph pad prism software .

ABTS Radical Cation Scavenging Activity:

ABTS radical cation assay was carried out using the method as described earlier (Khan et al.2012). 20mL of ABTS solution (7.4mM) used as a free- radical provider was treated with 20mL of Potassium persulphate (2.45mM) to produce free radicals. The solution was diluted with 98% of ethanol to obtain an absorbance of 1.5-2.4 at 734nm before used. The resulted ABTS reagent prepared using dilution was used in estimation process . 100µl of each fraction of plant extract (50µg/mL – 250µg/mL) was taken with 3ml of ABTS reagent in different test tubes and mixture was shaken thoroughly. The mixture was left in dark for 90min. After incubation absorbance was taken at 734nm using UV/VIS spectrophotometer . Ascorbic Acid was taken as positive control and was treated in the same manner as the plant extract . All tests were taken in triplicate.Percentage ABTS radical cation scavenging activity was calculated by the following formulae:Percentage ABTS radical cation scavenging activity= (A1 – A2 /A1) × 100. A1 = absorbance of control (ABTS solution without test sample) , A2 = absorbance of sample (ABTS solution with test sample)

Hydroxyl Radical Scavenging Activity:

The hydroxyl radical (OH) scavenging activity was evaluated by the method with some modifications (Obafemi et al.2017) . The reaction mixture contained 1mL of 0.75mM 1 mL of 10mM phenanthroline, 1mL of 0.75mMFerous Sulphate solution (FeSO4) and 1mL of 0.01% (v/v) hydrogen peroxide (H_2O_2) solution and 1 ml of test sample . 1mL of test sample was taken from the different concentrations of plant extract made from 50µg/mL to 250µg/mL. 1ml of double distilled water, 1mL of phenanthroline, and 1ml of Ferrous Sulphate solution was mixed well and were taken as Abs.0. The mixture was inculcated at 37°C for 30mins. After incubation absorbance was taken at 536nm using UV/Visible spectrometer . Ascorbic Acid was taken as positive control and was treated in the same manner as the test sample (plant extract). Deionized water was taken as blank.Percentage hydroxyl radical scavenging activity was calculated by the following formula: Percentage scavenging activity : (Abs. of sample- Abs. of blank / Abs.0 – Abs.of blank) × 100.

Nitric Oxide Scavenging Activity:

The nitric oxide scavenging activity was measured by method with slight modifications (Obafemi et al.2017). Sodium nitroprusside (10mM) solution was prepared in Phosphate buffer saline (PBS, pH 8.0). 0.5mL of different concentrations of plant extract (100µg/mL-500µg/ml) and 2.5ml of sodium nitroprusside was taken in test tubes. The reaction mixture was incubated at 25° C for 150mins. After incubation 1mL aliquot was taken from each fraction in different test tubes and 1mL of Griess Reagent was added to each fraction of mixture. Color change was observed and the absorbance was taken at 546nm using UV/VIS spectrophotometer. Ascorbic Acid was taken as positive control and was treated as the same way as that of fractions. 2mL of Sodium nitroprusside Solution made in phosphate buffer saline was taken as control. All the tests were done in triplicate. Percentage nitric radical scavenging activity was calculated by the following formula: %scavenging activity = (A0 – A1 / A0) × 100 . A0 = Abs. of control, A1= Abs. of plant extract fraction and Ascorbic Acid.

Iron Reducing power

Iron reducing power of plant extract was evacuated by the method of Medini et al. (2014). Different concentrations of plant extract were made from100µg/mL to 500µg/mL . 0.5mL of extract from each concentration was mixed with 0.5mL of phosphate buffer (200mmol/L) in test tubes. (The buffer pH was adjusted to pH 6.6.) . 2.5ml of 10% potassium ferrocyanide solution was added to each fraction of mixture and the reaction mixture was incubated at 50°C for 20mins. After incubation 2.5mL of 10% TCA (Trichloroacetic Acid) was added to the reaction fractions and was centrifuged at 650rpm for 10mins. After centrifugation 1mL from each fraction was taken in different test tubes and was mixed with 1ml of distilled water. 0.1mL of 0.1% Ferric chloride was added to each fraction. The absorbance was measured at 700nm using UV/ visible spectrophotometer. Ascorbic was taken as standard and was treated in the same as the test sample. The higher absorbance indicates the higher reducing power. The tests were performed in triplicate. The extract concentration that give 0.5 absorbance (IC50) was calculated from the graph of absorbance at 700 nm against the extract concentration.LogIC50 value was calculated by graph pad prism software.

Superoxide Radical Scavenging Activity :

Superoxide radical scavenging activity of each fraction of extract was determined by the nitro tetrazolium blue method (Khan et al.2012). Different concentrations of plant extract were made from 50µg/mL to 250µg/mL. The reaction mixture contained 1mL of nitro tetrazolium blue solution (NBT) , 1mL of nicotinamide adenine dinucleotide solution (NADH) and 0.1 mL of test sample (plant extract) of different concentrations. Further reaction was started by the addion of 100ul of phenazinemethosulfate solution (PMS) to each fraction of mixture. The reaction mixture was incubated at 25°C for 5min. The absorbance was taken at 560nm using the UV/ VIS spectrophotometer . The mixture containing all the reagent except the PMS solution was taken as blank . Ascorbic Acid was taken as positive control and Methanol was taken as negative control. All the assays were carried out in triplicate. % scavenging activity = (1- Abs. of sample/ Abs. of control) × 100.

Hydrogen Peroxide scavenging Activity:

The ability of plant extract to scavenge Hydrogen Peroxide was evaluated by the method of Ruch et.*al* with slight changes (Khan et al.2012). Hydrogen Peroxide solution (2mM) was prepared in Phosphate Buffer (50mM). Adjusted the pH of phosphate buffer to pH7.4. Different concentrations of plant extract were taken and their volume was made up to 0.8mL with Phosphate Buffer and were transferred into test tubes. 1200µl of Hydrogen Peroxide solution was added to each reaction mixture. Tubes were vortexed and the reaction mixture was left for 10min at room temperature. The absorbance was read at 230 nm by using an UV/VIS spectrophotometer. Phosphate buffer (pH 8.0) without Hydrogen Peroxide was taken as blank and Phosphate Buffer with Hydrogen Peroxide was taken as control. Ascorbic Acid was taken as positive control and was treated in the same manner as the plant extract. Percentage hydrogen peroxide radical scavenging activity was calculated by the formula: % scavenging activity: (1- Abs. of sample/ Abs. of control) × 100 .

Anti-Inflammatory activity of leaf extract of Eucalyptus globulus:

Protein denaturation was done according to the protocol describe by Gunathilake et al. (2018). The reaction mixture consisted of 0.4mL of 1% BSA , 4.78mL of phosphate buffer (pH 6.4) and different amount of *Eucalyptus globulus*leaf extract. The reaction mixture was incubated in water bath at 37° C for 15 mins. After the reaction mixture was heated at 70° C for 5 mins. The reaction mixture was immediately cool down. After cooling , the turbity was measured at 600nm using UV/VIS spectrophotometer . Phosphate buffer solution was taken as control. Aspirin (2-Acetoxybenzoic acid, Merck) was taken as positive standard. The percentage inhibition of protein denaturation was calculated by using the following formula.% inhibition of denaturation = 100 × (1- A2/A2) or% inhibition of denaturation = 100 - (A1-A2/A3). Where , A1 = absorbance of sample [prepared by mixing extract [50µL]+ BSA [400µL]+PBS [4.55mL], A2 = absorbance of the product control [prepared by extract [50µL]+ PBS [4.95mL], A3 = absorbance of the test control [prepared by mixing H2O [50 µl]=+ BSA [400µL]+PBS [4.55mL]

In addition, protein denaturant assay was also studied using fluorescent assay (Kronman and Holmes, 2008). The reaction mixture contained 0.4ml of 1% BSA, 4.78 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µLof *Eucalyptus globules* essential oil. The reaction mixture was incubated in water bath at 37°C for 15 min. After that reaction mixture was heated at 70°C for 5 min. Aftercooling, 1 mL of mixture was subjected to fluorescent spectroscopy analysis on Perkin Elmer Spectrophotometer (FL6500). The excitation wavelength was 280 nm and fluorescence emission spectrum was recorded over wavelength range from 300-400 nm. All experiments were done at room temperature (~30ºC).

In vitro Antibacterial activity

In vitro antibacterial activity of the *Eucalyptus globules* methanolicextract was carried out by agar disc diffusion method against test organism (gram-negative bacteria *Escherichia coli*, MTCC 40 and *Streptomycesaureus* MTCC 3160). Pathogens were purchased from Institute of Microbial Technology, Chandigarh. Sterile paper discs (6 mm in diameter) impregnated with 100 µl of *Eucalyptus globules*essential oil. Inoculums of both strains were prepared from 12-h

cultures, and OD of suspensions was adjusted to 0.5 at 570nm using spectrophotometer. A swab of bacteria suspension was spread on to the petri plates having Luria Broth and allowed to dry for 30 min. The discs with leaf extract (100 µL) were then applied and plates were left for 20 min at room temperature to allow to diffusion of extract followed by incubation at 37^0 C for 24 hours. After incubation, the extract diffuses into the luria agar plates and prevent germination and growth of the test microorganism and antibacterial activity was observed as the zone of inhibition around the discs. The experiment was repeated three times.

UV-VIS fingerprint analysis, FT-IR and Fluorescence spectroscopy analysis

UV-spectrophotometric analysis of extract was conducted using UV-VIS spectrophotometer (Labtronics) with slit width of 2nm, using a 10-mm cell at room temperature and were examined in the wavelength ranging from 200-400 nm. The peak values of the UV-VIS were recorded. FT-IR spectrometer was used to identify functional groups. A small amount of extract was taken in the sample cup of a diffuse reflectance accessory. The sample was scanned from 4000 to 400 $cm^{-1.}$ The peak values of the FT-IR were recorded. The fluorescence spectrum of sample was also measured. All experiments were done at room temperature (~30ºC).

RESULTS AND DISCUSSION

The leaf extract of *Eucalyptus globulus* shows considerate amount of phenol and flavonoid content present in it. The total phenolic content of the methanolic leaf extract of *Eucalyptus globulus* calculated from calibration curve of gallic acid is 50 (mg gallic acid equivalent per g dry weight) . The total flavonoid content of the methanolic leaf extract of *Eucalyptus globulus* is calculated from calibration curve of rutin is 23 (mg rutin equivalent per g dry weight).Phenolic compounds acts as reducing agents, which allow them to act as antioxidants. Their free radical scavenging activity is facilitated by hydroxyl groups , phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoid are plant secondary metabolite, their antioxidant activity depends upon the presence of OH group specially 3- OH. (Baba et al.2014) .

In – vitro Antioxidant activity of leaf extract of Eucalyptus globulus

DPPH radical scavenging activity is usually used to estimate the in – vitro antioxidant activity of natural compound or plant extract (Paulraj et al.2011). DPPH is a stable free radical that has been widely used in phytomedicine for the assessment of scavenging activity of bioactive fractions (Khan et al.2012). Figure1 shows the DPPH scavenging activity of *Eucalyptus globulus*leaves compared favourably with Ascorbic acid at all concentration tested. At the highest concentration (100ug/ml) the extract shows 80% inhibition while standard Ascorbic acid shows 85% inhibition. The results also showed that the extract exhibited a dose – dependent inhibition of DPPH radical. Log IC50 value showed in the Table.1 .

Scavenging capacities of various fractions of *Eucalyptus globulus*extract and Ascorbic acid was assessed by ABTS (2,2 azobis-(3-ethylbenzothiozoline-6-sulphonic acid) radical cation. ABTS is a blue chromophore produced by the reaction between potassium persulphatein the presence of plant extract or Ascorbic acid (Obafemi et al.2017) . In the present study as presented in Figure2 methanolic extract of *Eucalyptus globulus* shows remarkable ABTS scavenging activity as compared to Ascorbic acid. At the highest concentration (50ug/ml) the extract shows percentage inhibition 70.86% while standard Ascorbic acid shows 65.26% . LogIC50 value showed in the Table 1.

Among the oxygen radicals, Hydroxyl radical is an extremely reactive free radical which can

Table.1: logIC50 value of methanolic leaf extract of *Eucalyptus globulus*:

Estimations	LogIC50 value
DPPH Assay	1.309
ABTS radical scavenging activity	1.710
Hydroxyl radical scavenging activity	3.770
Nitric oxide radical scavenging activity	5.910
Iron reducing power	2.426
Superoxide radical scavenging activity	2.330
Hydrogen peroxide scavenging activity	0.2219

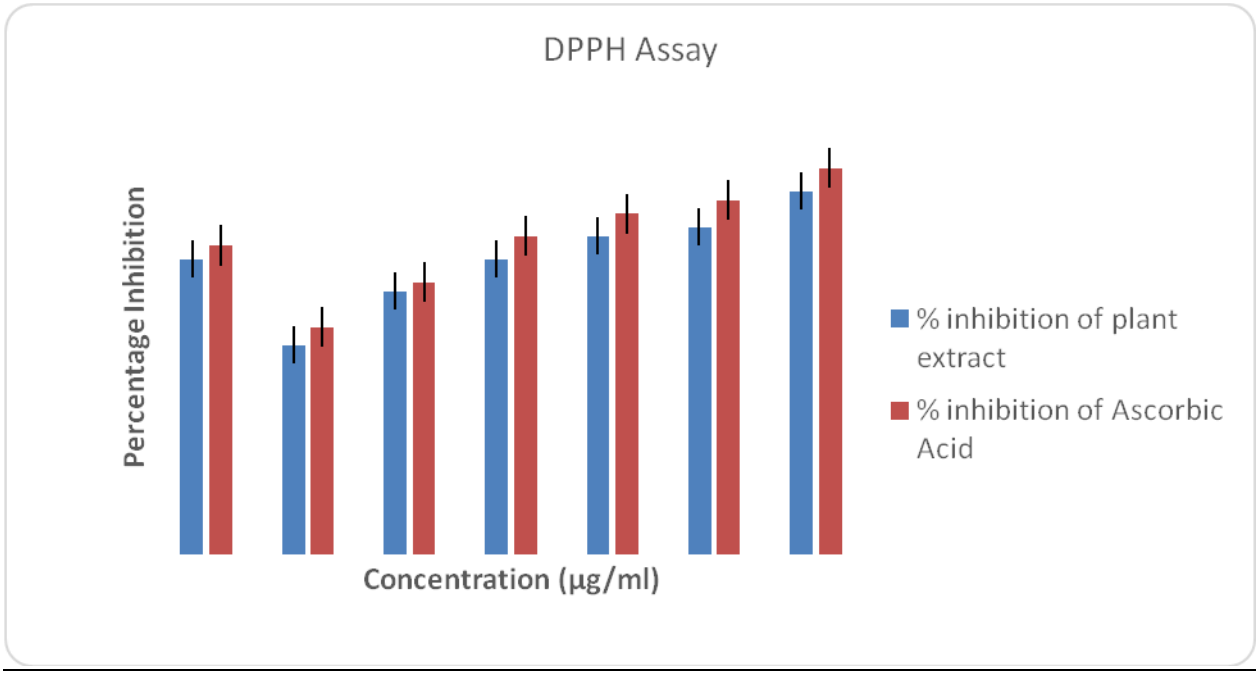

Figure.1: Percentage DPPH radical inhibition of methanolic leaf extract of *Eucalyptus globulus*

induces sever damage to the adjacent biomolecules such as protein , DNA and lipids causes lipids peroxidation. (Khan et al.2012) . The radical has the capability to form adducts with nucleotides in DNA and cause stand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity (Thirunavukkarasu et al.2011) . Figure3 shows the Hydroxyl scavenging activity of *Eucalyptus globulus* leaves compared favourably with Ascorbic acid at all

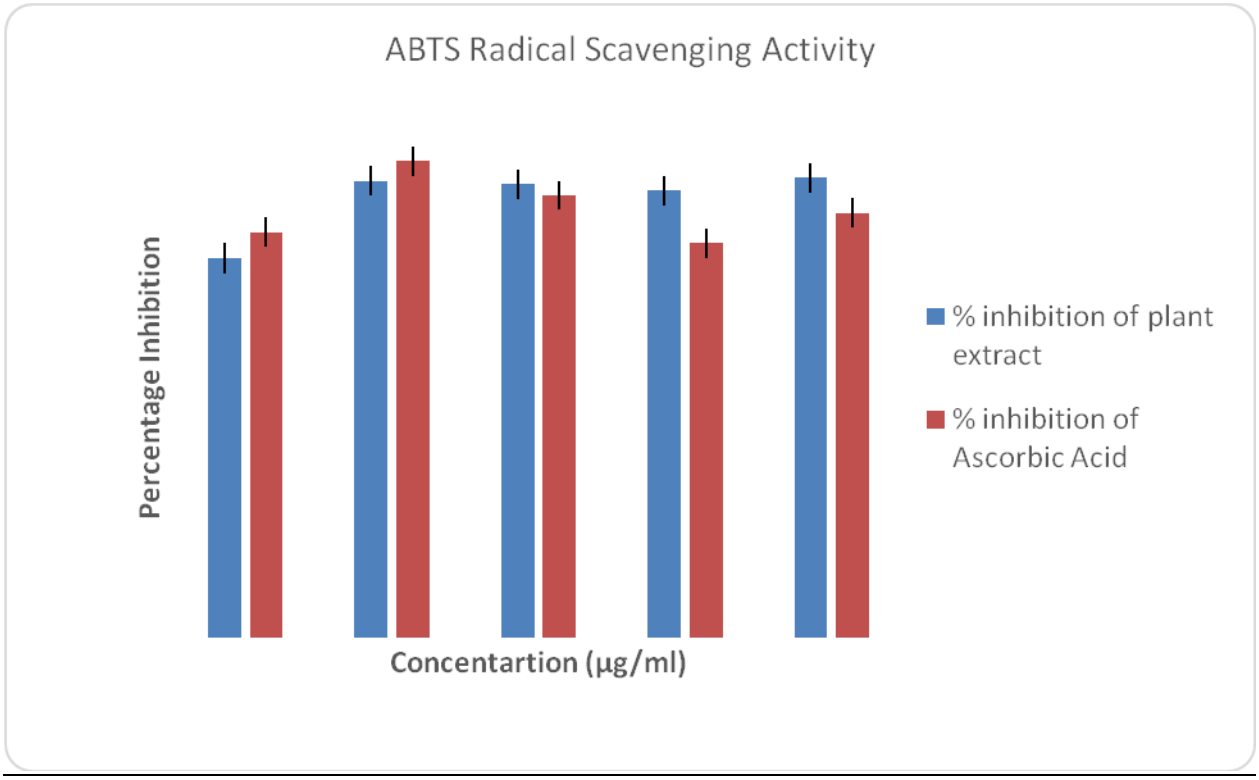

Figure.2 : Percentage ABTS radical inhibition of methanolic extract of *Eucalyptus globulus* .

concentration tested. At the highest concentration (250ug/ml) the extract shows 86.30% inhibition while standard Ascorbic acid shows 91.26%. LogIC50 value showed in the table.2 . Nitric oxide is a free radical produced in mammalian cells which is involved in the regulation of various physiological processes including neurotransmission, vascular homeostasis ,anti microbial and antitumor activities. However, excess of NO is associated with diseases (Obafemi et al.2017). Figure.4 shows the *Eucalyptus globule* sextract showed a dose – dependent increase in nitric oxide scavenging activity. At the highest concentration (500ug/ml) the extract shows percentage inhibition 64.19% while standard Ascorbic acid shows 89.70%. LogIC50 value showed in the Table 1.

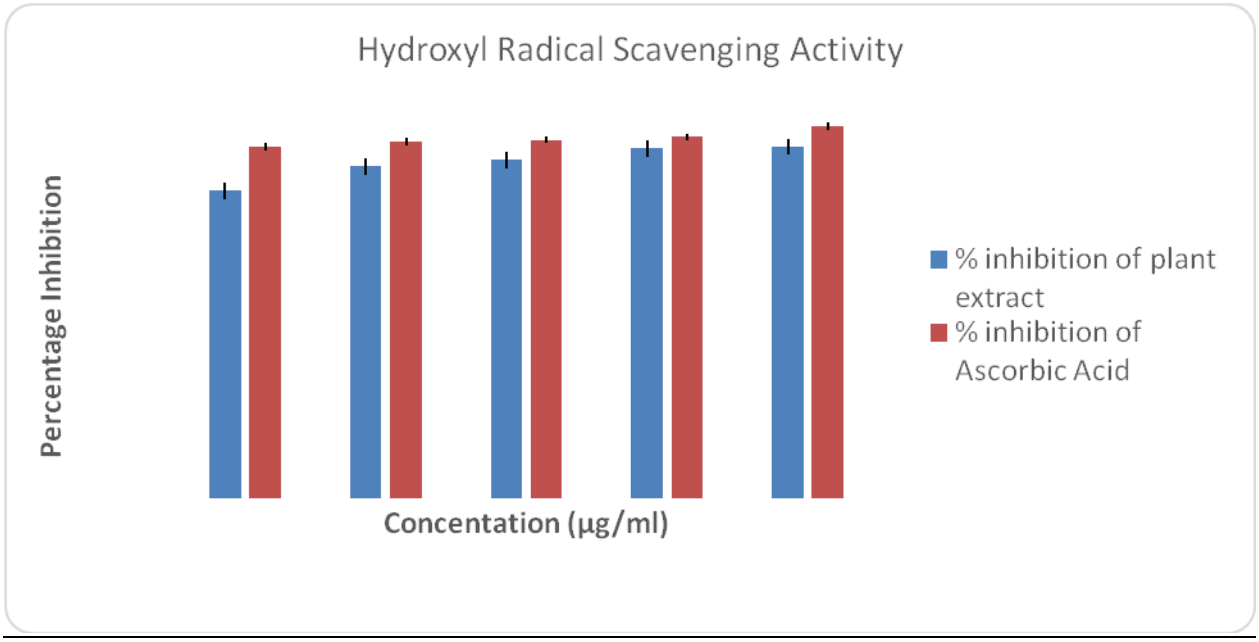

Figure.3: Percentage hydroxyl radical scavenging activity inhibition of methanolic leaf extract of Eucalyptus globulus.

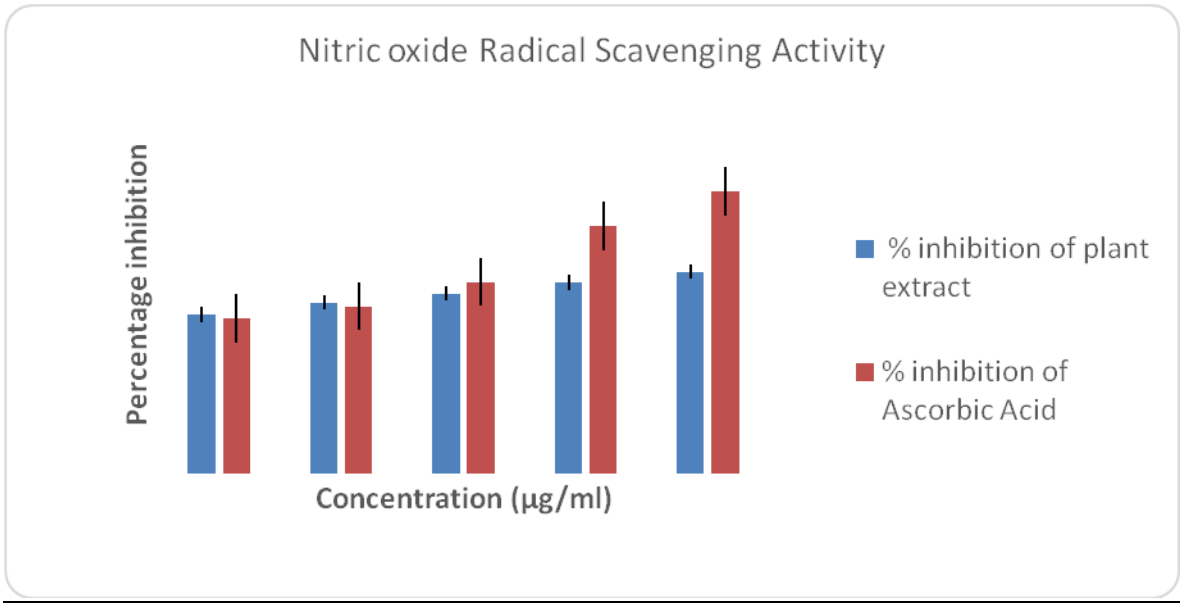

Figure.4 : Percentage nitric oxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus* .

Iron reduction is often used as an indicator of electron donating activity , which is an important mechanism of phenolic oxidants . The reducing ability of a compound depends upon reductones (antioxidants) , which exert thye antioxidant activity by breaking the free radical chain by donation a hydrogen atom. Figure 7 depicts the reductive capability of leaf extract of Eucalyptus globulus. Reducing power of extract increases with the increase in concentration of extract . Thus, higher the concentration higher is the reducing power or reductive potential. The leaf extract of Eucalptus globulus showed good reducing power ability in dose dependent manner which was comparable with standard Ascorbic acid .

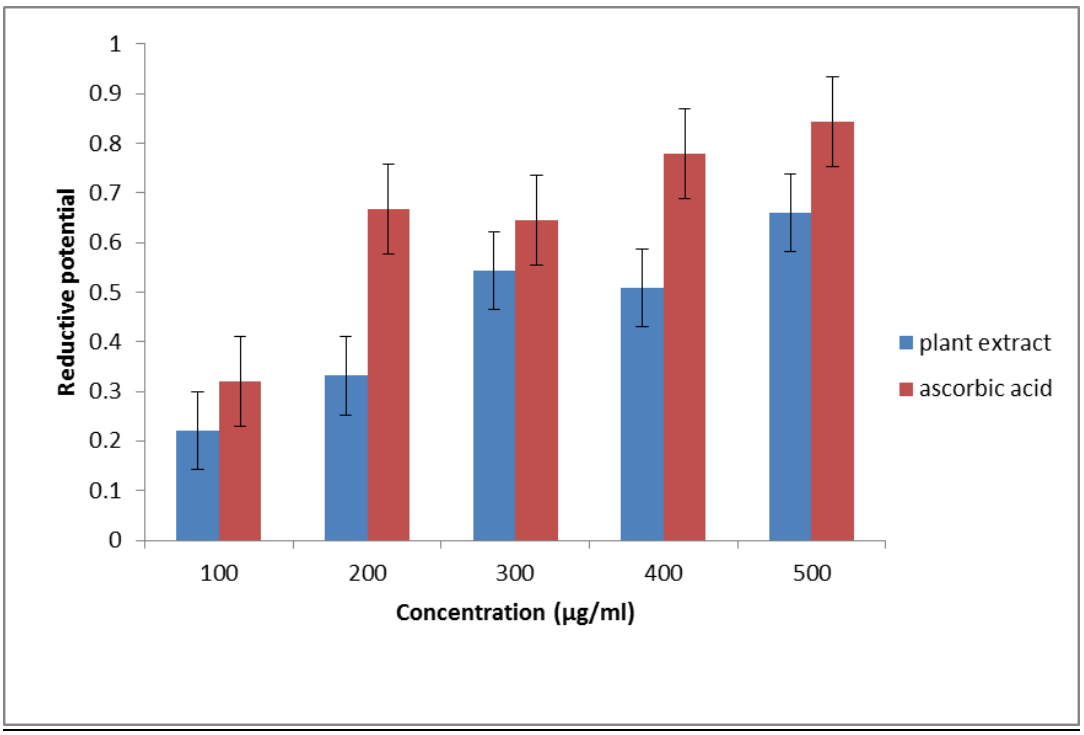

Figure .5 : Reductive potential of methanolic leaf extract of *Eucalyptus globulus* .

Superoxide anion is a weak oxidant produced during various biological reactions are highly toxic .Superoxide anion is known as an important initial radical and plays an important role in the formation of other oxygen-species , such as hydrogen peroxide or single oxygen . Superoxide is generate in vivo by several oxidative enzymes , including xanthine. The results show that leaf extract of *Eucalyptusglobulus* has a potent superoxide scavenging activity which is due to the presence of antioxidants compounds . In this study , Figure6 shows the superoxide scavenging activity of *Eucalyptusglobulus* leaves compared favourably with Ascorbic acid at all concentration test . As the concentration of leaf extract of Eucalyptus globulus increases the percentage inhibition also increases along with the Ascorbic acid . Higher the concentration , higher will be the percentage inhibition . At the highest concentration (500ug/ml) the extract shows percentage inhibition 14.89% while standard Ascorbic acid show 33.19% .

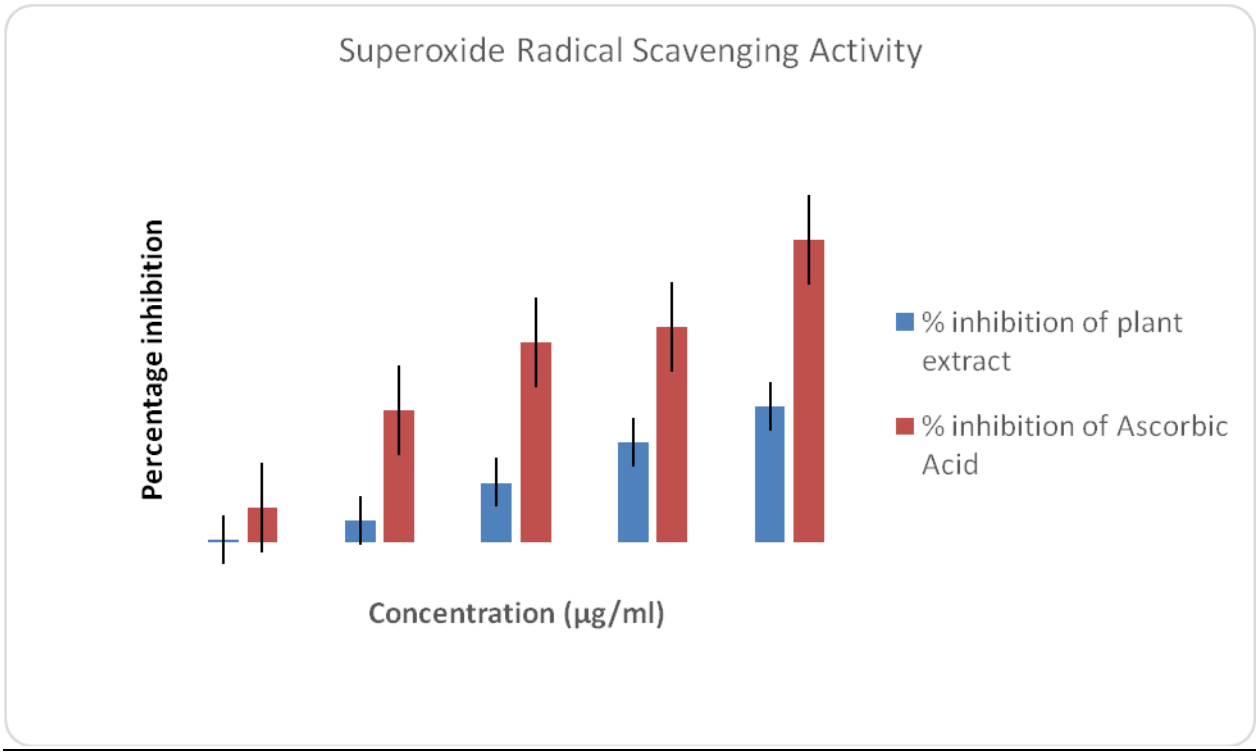

Figure.6: Percentage superoxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus.*

Hydrogen Peroxide is highly reactive because of its ability to penetrate into biological membranes . H2O2 itself is not very reactive , but it can sometimes be toxic to cells because it may give rise to hydroxyl radicals in cells . The results show that leaf extract of *Ecalyptusglobulus*has a potent H2O2 scavenging activity which is due to the presence of antioxidants compounds . As the antioxidant components present in the leaf extract are good electron donors , they may accerlate the conversion of H2O2 to H2O . In this study , Figure 7 shows the hydrogen peroxide scavenging activity of *Eucalyptusglobulus* leaves compared favourably with Ascorbic acid at all concentration test . As the concentration of leaf extract of Eucalyptus globulus increases the percentage inhibition also increases along with the Ascorbic acid . Higher the concentration , higher will be the percentage inhibition . At the highest concentration (500ug/ml) the extract shows percentage inhibition 84% while standard Ascorbic acid show 64% .

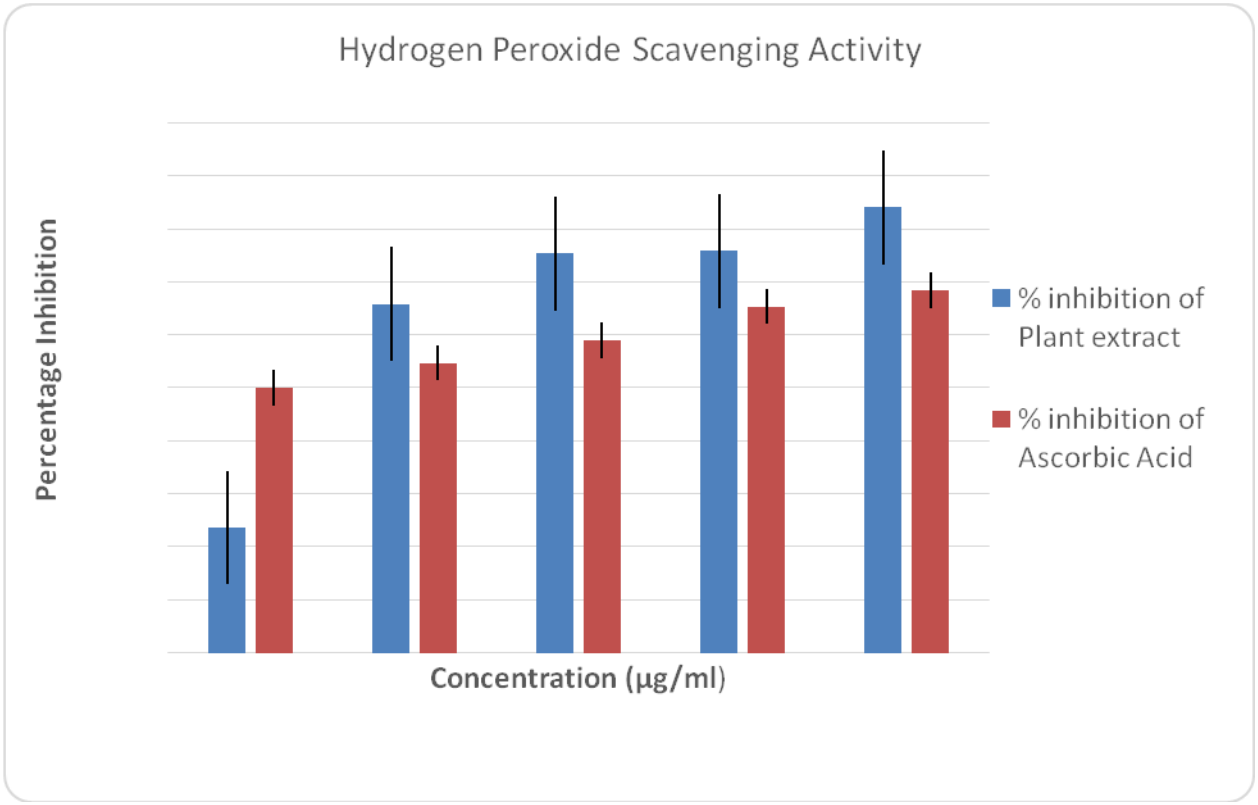

Figure.7: Percentage hydrogen peroxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus*.

The antioxidant values of herbal derivatives are linked with each other and herbal products may contain a variety of ingredients with different antioxidant properties (Khan et al., 2012). We therefore opine that the marked antioxidant activities of extract may be attributed to the presence of flavonoids and other phenolic compounds as observed in this study. Richness of antioxidant activities of any bioactive compound is the symbol of its potential use as food/drug supplement to control damage of biomolecules by inhibiting free radicals in biological system and consequently rejuvenates the body functions (Hayat et al., 2017). Flavonoids and phenolics, being polyphenolics, are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants

Anti- inflammatory activity of leaf extract of Eucalyptus globulus

Denaturation of protein is the major cause of inflammation. As shown in the Figure.10methanolic leaf extract of *Eucalyptus globulus*shows a remarkable anti-inflammatory potential along with drug Aspirin in dose dependent manner . The results , clearly shows the leaf extract of *Eucalyptus globulus*effectively inhibit the denaturation of BSA in a comparable manner and significant manner same as the drug Aspirin . Higher the concentration dose of leaf extract of plant *Eucalyptus globulus* higher will be the anti-inflammatory activity . It can therefore be concluded , that leaf extract of *Eucalyptus globulus*possess significant anti-inflammatory activity. Results indicated that these anti-inflammatory activities occur due to the presence of bioactive compounds , such as flavonoids , polyphenols and carotenoids. This property of *Eucalyptus globulus*is very effective and due to this *Eucalyptus globulus*can be used an herb plant .

In vitro antimicrobial activity

Eucalyptus methanolic extract was quantitatively assessed for *in vitro* antimicrobial activity using agar disc diffusion method. The extract depicted strong antimicrobial activity against E coli (Figure 9, Table 2). Consequently, the antimicrobial activities of *Eucalyptus* extract may be attributed to synergistic effects of polyphenolics.Our findings were in consonance with the earlier studies (Siramon and Ohtani, 2007). It was noteworthy observation as mostly literature

suggests that gram negative bacterial are more resistant than gram positive due to complex nature of cell wall (Siramon and Ohtani, 2007). Extract from eucalyptus species has been reported to have antibacterial activity due to presence of bioactive molecules like Eucalyptol, citronella (Swamy et al ., 2016). Phenolics are reported to be involved in the inhibition of various metabolic and biosynthetic pathways.

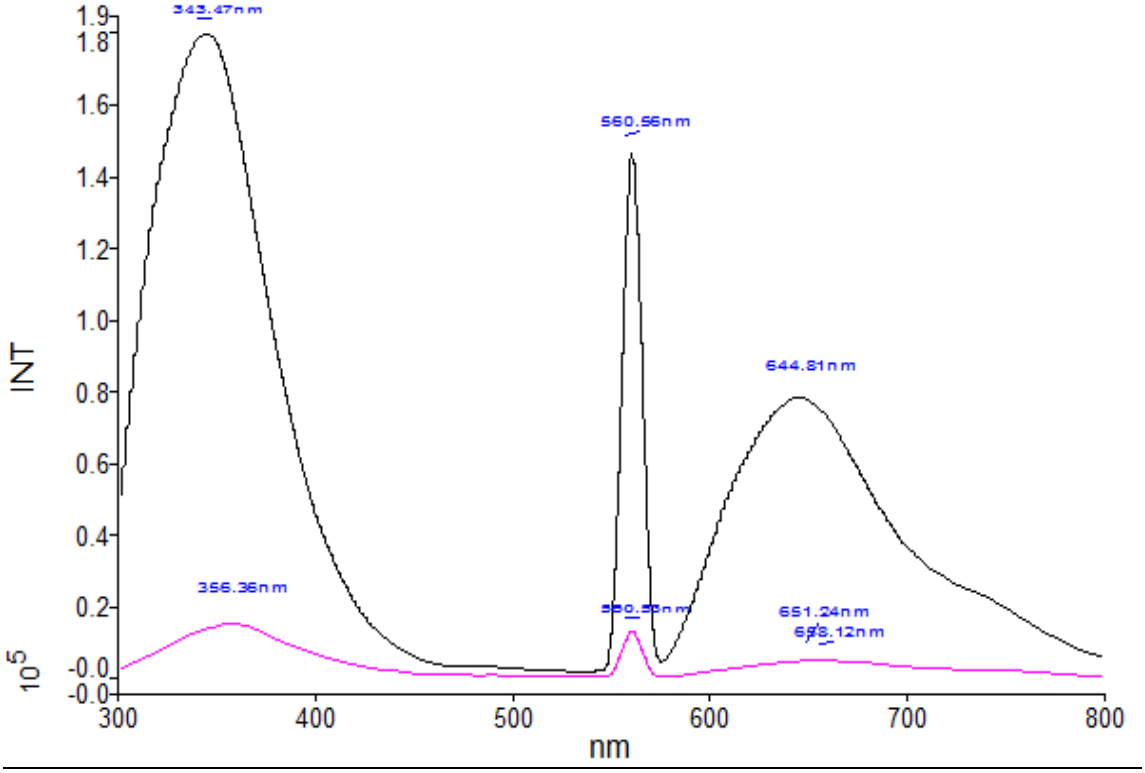

Figure.8 shows the anti-inflammatory activity of leaf extract of *Eucalyptus globulus* .

Table 2: Antimicrobial analysis of essential oil

Strain	**Strain type**	**Zone of inhibition (cm)**	
		C	**ME**
Escherichia coli	Gram negative	1.8	2.2

Here: C= Positive control (Vancomycin antibiotic, 10mg), ME= extract

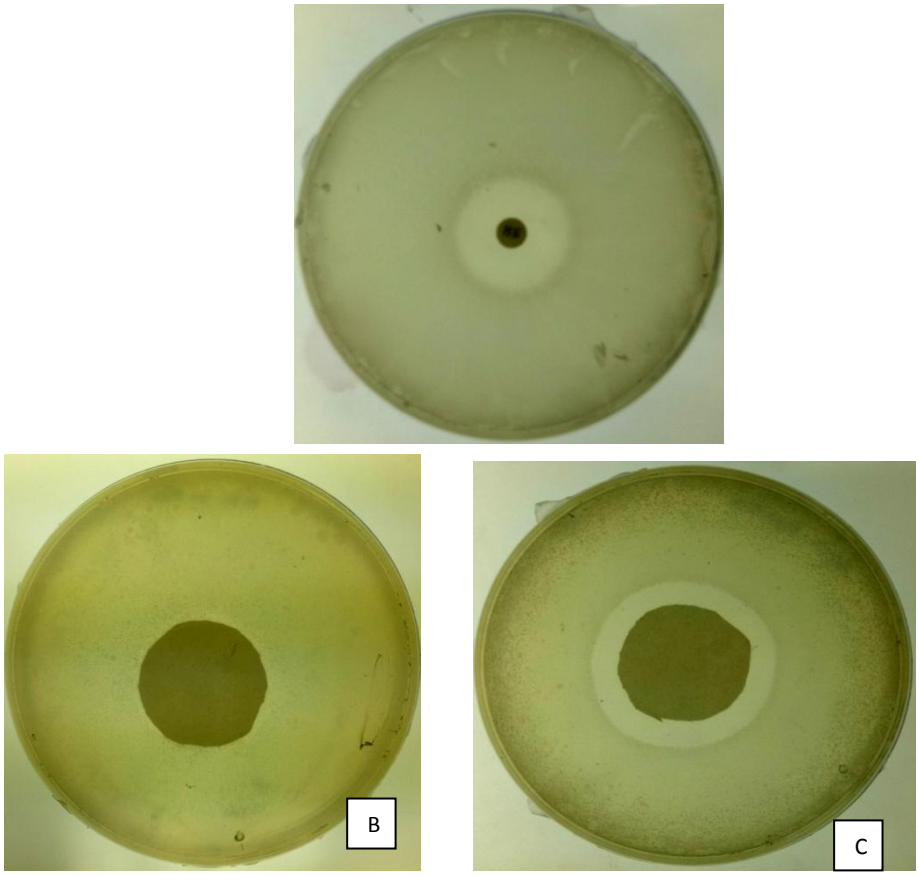

Figure 9. Antibacterial property of Eucalyptus globulus leaf extract against *E. coli* (MTCC 40) A = Vancomycin 30 Mg (Positive control), B= Blank (solvent), C= extract (100 µL),

CONCLUSION

Based upon the results obtained in the present study , it is concluded that the methanolic leaf extract of *Eucalyptus globulus* contains the considerate amount phenols and flavonoids. It is also exhibit high antioxidant and free radical scavenging activities relevant to wound treatment. It also has reducing power . This also showed that the leaf extract of *Eucalyptus globulus* has a anti-inflammatory property which indicates the presence of bio- active compounds in the extract of *Eucalyptus globulus* . This indicates that *Eucalptus globulus* plant is a significant source of

natural antioxidant , which might be helpful in preventing the progress of various oxidative stresses and treating wounds . However , isolation of bioactive compounds would assist to ascertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses .

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