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Phytochemicals, *In-Vitro* Antioxidant, *In-Vitro* antibacterial and Anti-inflammatory activity of methanolic leaf extract from *Eucalyptus globulus.*

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Abstract: Background: *Eucalyptus globulus* is one of the important medicinal plant widely used. *Eucalyptus globulus* plant belongs to genus *Eucalyptus* and family Myrtacaea. Genus *Eucalyptus* includes more than 700 species which are successfully introduced widely. In this study methanolic leaf extract of *Eucalyptus globulus* was studied for the estimation of photochemical constituents, in-vitro antioxidant and anti-inflammatory activity. Methods: Phytochemical constituents are checked by total phenol content estimation and total Flavonoids content estimation. UV-VIS, FT-IR and fluorescents analysis was also conducted. While antioxidant activities of plant extract was checked through scavenging of various free radical. For anti- inflammatory, checked by BSA protein denaturation assay. *In vitro* antimicrobial activity was also monitored. Results: Phytochemical screening revealed the presence of flavonoids, phenols, carbohydates and saponins. The leaf extract of *Eucalyptus globulus* shows considerate amount of phenol and flavonoid content present in it. The total phenolic content of methanolic leaf extract of *Eucalyptus globulus* calculated from calibration curve of gallic acid is 9.4mg/20g (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 14mg/20g (mg rutin equivalent per g dry weight). The reducing power assay showed the reducing ability of the leaf extract were significantly increased as the concentration increased. Extract showed anti-inflammatory and anti-bacterial potential in it. Conclusion: This study concluded that methanolic extract *Eucalyptus globulus* possesses potent antioxidant and anti-inflammatory activity possibly due to good quantity of flavonoid and phenolic content present in it.
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Introduction

Literature reveals that even today in western medicine and despite the progress in synthetic chemistry, plants are the backbone of primary health care and approximately 80% of population relies on plants. Around 700,000 plant species are widely used in traditional and modern medicine (Kumar et al., 2018). Currently researches are focusing immensely on the protective effects against oxidative stress acquired from natural antioxidants. Plants are rich sources of natural antioxidants like phenolic acid, flavonoids and anthocyanins which constitute an important class of antioxidants which can help to fight the formation of free radicals in the human body and thus slow down the cellular aging and damaging process. According to the incredible results obtained, interest are more accumulated in bioactive compounds extracted from medicinal plants than the synthetic drugs which were restricted due to their side effects. Polyphenol and flavonoids used as natural antioxidants which are obtaining importance due to their benefits in human health and also for decreasing the risks of degenerative diseases by the reduction of oxidative stress (Khan et al., 2012). The medicinal value of plant mainly describes by the amounts of presence of phytochemical constituents and antioxidants present in the plant. The higher the number, the larger their medicinal value. Even natural antioxidants are more beneficial than the synthetic due to their safety concerns. These factors have inspired the widespread screening of plants for possible medicinal and antioxidants properties, the isolation and characterization of diverse phytochemicals and the development and the utilization of antioxidant of natural origin (Michalak. 2006). It is believed that the phytochemical composition and the in-vitro

antioxidant activity of plant might give a clue to its therapeutic potential.

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 eucalyptus and family Myrtacaea. 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 has various concentrations of minerals. *Eucalyptus* essential oil is colourless and has a distinctive taste and odour and typical volatility. Essential oil of *Eucalyptus* is highly flammable and contains compounds that are natural disinfectants and pest deterrents. Essesntial oil of *Eucalyptus globulus* is composed of volatile organic compounds including hydrocarbons, alcohols, aldehydes, ketones, acids, ethers and esters (Su et al., 2006). Most of the compounds are monoterpenes and sesquiterpenes in nature which consist of two or more isoprene unit (C5H8). 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 *Eucalyptus globulus* 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 globulus* leaf extracts and its characterization is still not well known. Thus the main objective of present work is to arrange the fraction of *Eucalyptus globulus* for the determination of phytochemical constituents, its characterization by UV, FT-IR and florescent techniques and to evaluate its antioxidant and antimicrobial potential.

Material and methods

Sampling site

Eucalyptus globulus plant leaves were collected from the surroundings of village Sofi Pind, Jalandhar, Punjab (Figure.1). The plant was identified at Dept of Botany and voucher with number BT101 was deposited in herbarium, of Dept of Biotechnology. All the leaves were very healthy and were disease free so used for further analysis.

Extraction and sample preparation:

The extract of *Eucalyptus* plant tissue was prepared in 80% Methanol. 10g of fresh leaves of plant *Eucalyptus globules* was taken and washed, dried and 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

The eight chemical standard solutions were prepared in different solvents. Glutathione Reduced and Glutathione Oxidized both were prepared in distilled water. Rutin and 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.

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

DPPH Radical Scavenging Activity:

Chemicals used:

a) DPPH (1,1-diphenyl-2-picryl-hydrazyl).

b) Methanol

c) Ascorbic Acid

Procedure: 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: Percentage scavenging activity = (Abs. of control - Abs. of fraction / Abs. of control) × 100. LogIC50 value was calculated by graph pad prism software.

ABTS Radical Cation Scavenging Activity:

Chemicals used:

a) ABTS (2,20-azinobis-(-3-ethylbenzothiamine)-6-sulphonic Acid)

b) Potassium Persulphate

c) Methanol

d) Ascorbic Acid

Procedure: ABTS radical cation assay was carried out using the method of Re et *al* (45 (Khan et al.2012). 20ml of ABTS solution (7.4mM) was 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. 100ul 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. LogIC50 value was calculated by graph pad prism software.

Where,

A1 = absorbance of control (ABTS solution without test sample)

A2 = absorbance of sample (ABTS solution with test sample)

Hydroxyl Radical Scavenging Activity:

Chemicals used:

a) 1, 10 Phenanthroline

b) Ferous Sulphate

c) Hydrogen Peroxide

d) Ascorbic Acid

Procedure: The hydroxyl radical (OH) scavenging activity was evaluated by the method of Jin et. *al. (1996)* with some modifications (Obafemi et al.2017). The reaction mixture contained 1ml of 0.75mM 1,10 phenanthroline, 1ml of. 0.75mMFerous Sulphate solution (FeSO4) and 1ml of 0.01% (v/v) hydrogen peroxide (H2O2) solution and 1 ml of test sample. 1ml of test sample was taken from the different concentrations of plant extract made (50µg/ml-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.LogIC50 value was calculated by graph pad prism software.

Nitric Oxide Scavenging Activity:

Chemicals used:

a) Sodium nitroprusside

b) Phosphate Buffer Saline (PBS)

c) Hydrochloride

d) Ascorbic Acid.

Procedure: The nitric oxide scavenging activity was measured by the modified method of method of Sreejevan and Rao (1997) with slight modification (Obafemi et al. 2017). Sodium nitroprusside (10mM) solution was prepared in Phosphate buffer saline (PBS). 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 (1% sulphanilamide (w/v), 2%Phosphoric Acid (v/v), 0.1%, naphthylethylene diamine hydrochloride (w/v)) was added to each fraction of mixture. Colour 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:

Percentage scavenging activity = (A0 – A1 / A0) × 100.

Where,

A0 = Abs. of control.

A1= Abs. of plant extract fraction and Ascorbic Acid.

Iron Reducing power

Chemicals used:

a) Potassium ferrocyanide

b) Ferric Chloride.

c) Trichloroacetic acid

d) Phosphate Buffer

e) Ascorbic Acid

Procedure: Iron reducing power of plant extract was evacuated by the method of Medini et al. 2014). Different concentrations of plant extract were made (100µg/ml-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 gave 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.

6. Superoxide Radical Scavenging Activity:

Chemicals used:

a) NBT solution (nitroblue tetrazolium)

b) NADH solution (nicotinamide adenine dinucletide)

c) PMS solution (phenazinemethosuphate)

d) Phosphate buffer

Procedure: 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 (50µg/ml-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 phenazine methosuphate 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 and both the controls were treated in the same manner as the test sample or plant extract. All the assays were carried out in triplicate. Percentage superoxide radical scavenging activity was calculated by the formula: Percentage scavenging activity: (1- Abs. of sample/ Abs. of control) × 100.LogIC50 value was calculated by graph pad prism software.

Hydrogen Peroxide scavenging Activity:

Chemicals used:

a) Hydrogen Peroxide solution

b) Phosphate Buffer

c) Ascorbic Acid

Procedure: 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. 1200ul of Hydrogen Peroxide solution was added to each reaction mixture. Tubes were vortex and the reaction mixture was left for 10min at room temperature. The absorbance was taken at 230nm with the help of UV/VIS spectrophotometer. Phosphate buffer 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: Percentage scavenging activity: (1- Abs. of sample/ Abs. of control) × 100.LogIC50 value was calculated by graph pad prism software,

Anti-Inflammatory activity of leaf extract of Eucalyptus globulus:

Chemicals used:

a) Bovine albumin serum (BSA) 5%

b) Aspirin (Drug) in pure form

c) Phosphate Buffer saline PH 6.4

Procedure: Protein denaturation was done according to the protocol describe by Gunathilake et al.2018 with some modifications. 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 minutes. After the reaction mixture was heated at 70° C for 5 minutes. 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 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) Where, A1 = absorbance of control sample. A2 = absorbance of the test sample Or % inhibition of denaturation = 100 - (A1-A2/A3)

Where, A1 = absorbance of sample [prepared by mixing extract [50UL]+ BSA [400UL]+PBS [4.55ML], A2 = absorbance of the product control [prepared by extract [50UL]+ PBS [4.95ML], A3 = absorbance of the test control [prepared by mixing H2O [50ul]=+ BSA [400UL]+PBS [4.55ML]

In addition, protein denaturant assay was also studied using fluorescent assay. The reaction mixture contained 0.4ml of 1% BSA, 4.78 mL of phosphate

buffered saline (PBS, pH 6.4) and 100 ul of *Eucalyptus globulus* essential oil. The reaction mixture was incubated in water bath at 370 C for 15 min. After that reaction mixture was heated at 700 C for 5 min. After cooling, 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).

Total Phenolic Content:

Chemicals used:

a) Folin Ciocalteus Reagent

b) Sodium carbonate

c) Gallic Acid

Procedure: Total Phenolic content of Eucalyptus plant extract was calculated Spectrometerically 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. 200ul 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% Folin Ciocalteus 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. 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:

Chemicals used:

a) Rutin

b) Aluminium Chloride

c) Sodium Nitrite

d) Sodium Hydroxide

Procedure: The Total Flavonoid content of crude extract was determined by Aluminium Chloride colorimetric method (Baba et al.2015). 50ul 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 were 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 Antibacterial activity

In vitro antibacterial activity of the *Eucalyptus globules* extract was carried out by agar disc diffusion method against test organism (gram-negative bacteria *Escherichia coli,* MTCC 40 and *Streptomyces aureus* MTCC 3160). A swab of bacteria suspension was spread on to the petri plates having Luria Broth. Sterile paper discs (6 mm in diameter) impregnated with *Eucalyptus globules* extract were placed on culture plates. Nanoemulsions without essential oil were taken as negative control while standard vancomycin (30 mg) discs were served as positive control. The plates were incubated at 37^0 C for 24 hours.

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 was used to identify functional groups. A small amount of extract was taken in the sample cup of a diffuse reflectance accessory. IR spectrum was obtained using FT-IR infrared spectrophotometer (Perkin Elmer, USA spectrophotometer). The sample was scanned from 4000 to 400 $cm^{-1.}$ The peak values of the FTIR were recorded. The fluorescence spectrum of sample was measured on Perkin Elmer Spectrophotometer (FL6500). All experiments were done at room temperature (~30ºC).

Results and discussion

Preliminary phytochemical screening of methanolic leaf extract of *Eucalyptus globus* revealed the presence of phenols, flavonoids, saponins and carbohydrates compounds (Table 1). 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 9.4mg/20g (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 14mg/20g (mg rutin equivalent per g dry weight) (Table. 3). Phenolic compounds have redox properties, 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).

UV-VIS analysis of methanolic extract was scanned in the wavelength ranging 200-800 nm using the UV-VIS spectrophotometer and characteristic peaks were detected (Fig. 1). The UV- spectra was performed to indentify the Bioactive compounds containing π-bonds, σ-bonds and lone pair of electrons, chromrophores and aromatic rings. The qualitative UV Spectroscopy profile of extract revealed different sharp peaks from 200-220 nm with absorbance of 2.0-2.5 (Fig.1), indicating the accumulation of secondary metabolites. The florescent emission spectra of extract is shown in Fig. 2. It was cited that various bioactive components of extracts when excited with suitable light emit fluorescence. Notably sharp peaks were detected, indicating that at least two different florescent substances possibly presented in the eucalyptus methanolic extract (Mylle et al., 2013). The FT-IR spectrum profile was illustrated in Figure. 2. The FT-IR gave two peaks whose values are 3310.38 and 1636.13. The peak obtained at 3310.38 indicated the presences of O-H stretching group means it contained hydroxyl group. The peak obtained at 1636.13 indicated C=C stretching group which illustrated that it contained alkane group. Hydroxyl group and Alkane compound group considered to be secondary metabolites. Thus, it indicated that leaf extract of *Eucalyptus globulus* contained secondary metabolites and can be helpful in wound treatment.

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). Figure 3 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. 2.

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 persulphate in the presence of plant extract or Ascorbic acid (Obafemi et al.2017). In the present study as presented in Figure 4 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 table2.

Among the oxygen radicals, Hydroxyl radical is an extremely reactive free radical which can 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). Figure 5 shows the Hydroxyl scavenging activity of *Eucalyptus globulus* leaves compared favourably with Ascorbic acid at all 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 neurotrasmission, vascular homeostasis, anti microbial and antitumor activities. However, excess of NO is associated with diseases (Obafemi et al. 2017). Figure.6 shows the *Eucalyptus globulus* extract 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 in the table 2.

Iron reduction is often used as an indicatior 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.

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 *Eucalyptus globulus* has a potent superoxide scavenging activity which is due to the presence of antioxidants compounds. In this study, Figure 8 shows the superoxide scavenging activity of *Eucalyptus globulus* 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%.

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 *Ecalyptus globulus* 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 9 shows the hydrogen peroxide scavenging activity of *Eucalyptus globulus* 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%.

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.10 methanolic 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 their 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 a herb plant.

In vitro antimicrobial activity

Eucalyptus methanolic extract was quantitatively assessed for *in vitro* antimicrobial activity using agar disc diffusion method. Our findings were in consonance with the earlier studies (Siramon and Ohtani, 2007). In the present study, extract from Eucalyptus showed strong antimicrobial activity. 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.

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.

Table 1. shows the results of preliminary phytochemical screening conducted with sample of methanolic leaf extract of *Eucalyptus globulus*:

Serial number	Test name	Result
1.	Test for terpenoids	Negative
2.	Test for phenols	Positive
3.	Test for saponins	Positive
4.	Test for carbohydrate	Positive
5.	Test for Amino acid	Negative
6.	Test for proteins	Negative
7.	Test for glycosides	Negative
8.	Test for flavonoids	Present

Table 2 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

Table 3 Total phenol and flavonoid content of methanolic leaf extract of *Eucalyptus globulus*:

Total phenolic content	9.4mg/20g (mg gallic acid equivalent per g dry weight)
Total flavonoid content	14mg/20g (mg rutin equivalent per g dry weight)

Fig: Pictorial view of plant (*Eucalyptus globulus*) used in this study

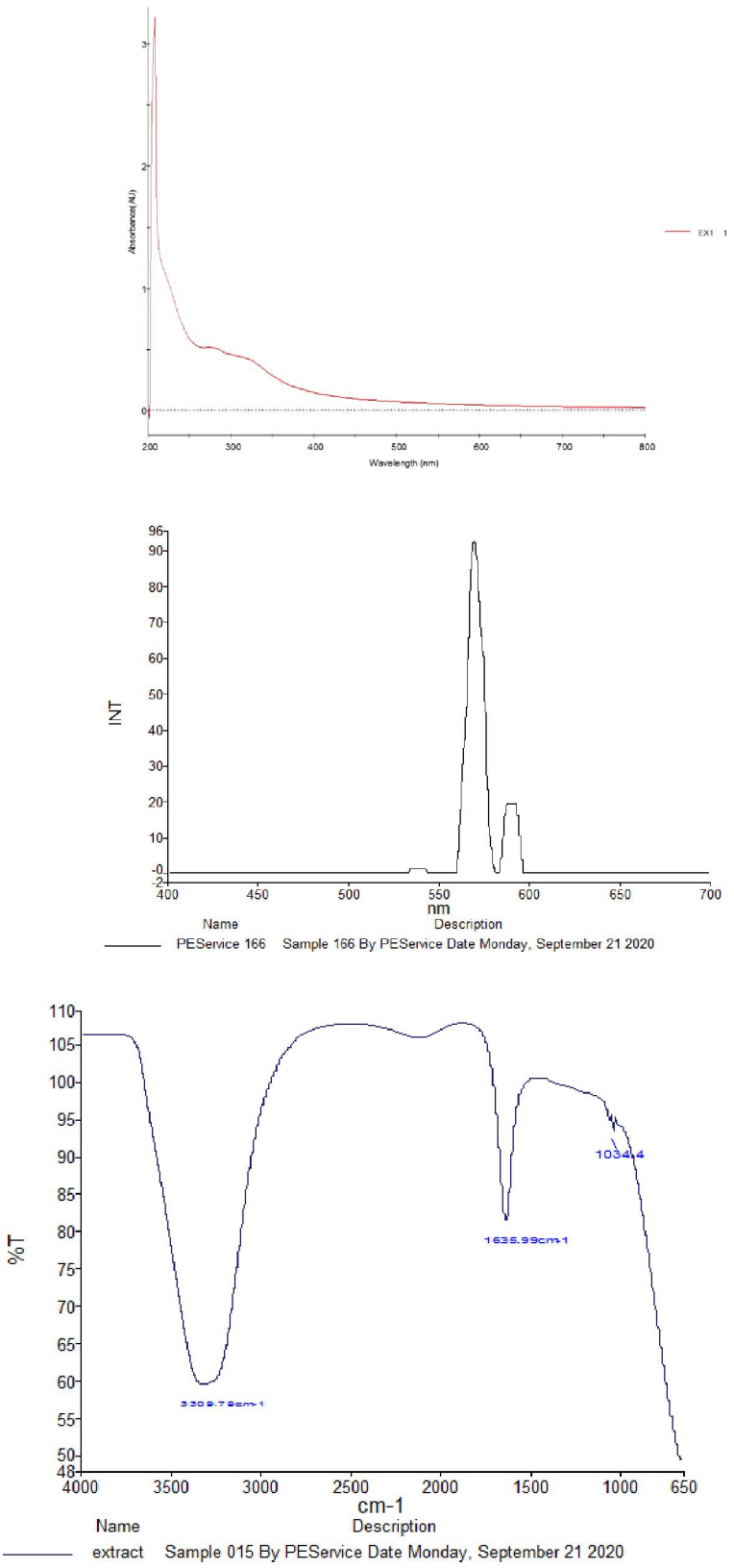


Fig 2: UV-VIS, fluorescent and FT-IR analysis of extract

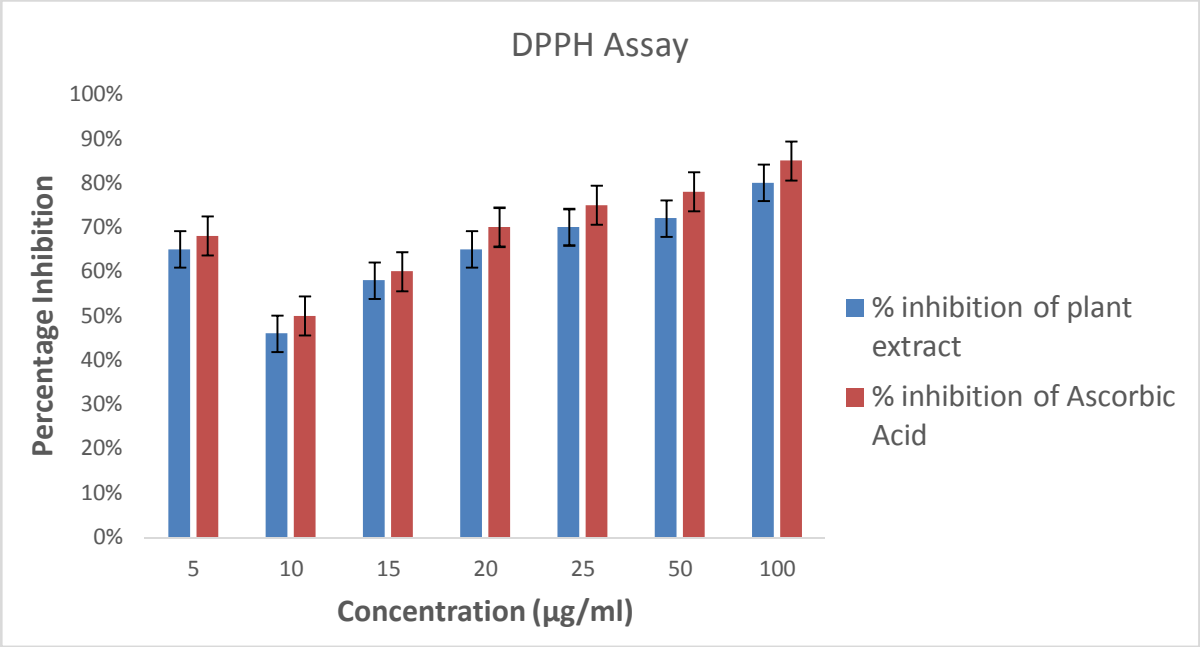


Figure 3: Percentage DPPH radical inhibition of methanolic leaf extract of *Eucalyptus globulus*.

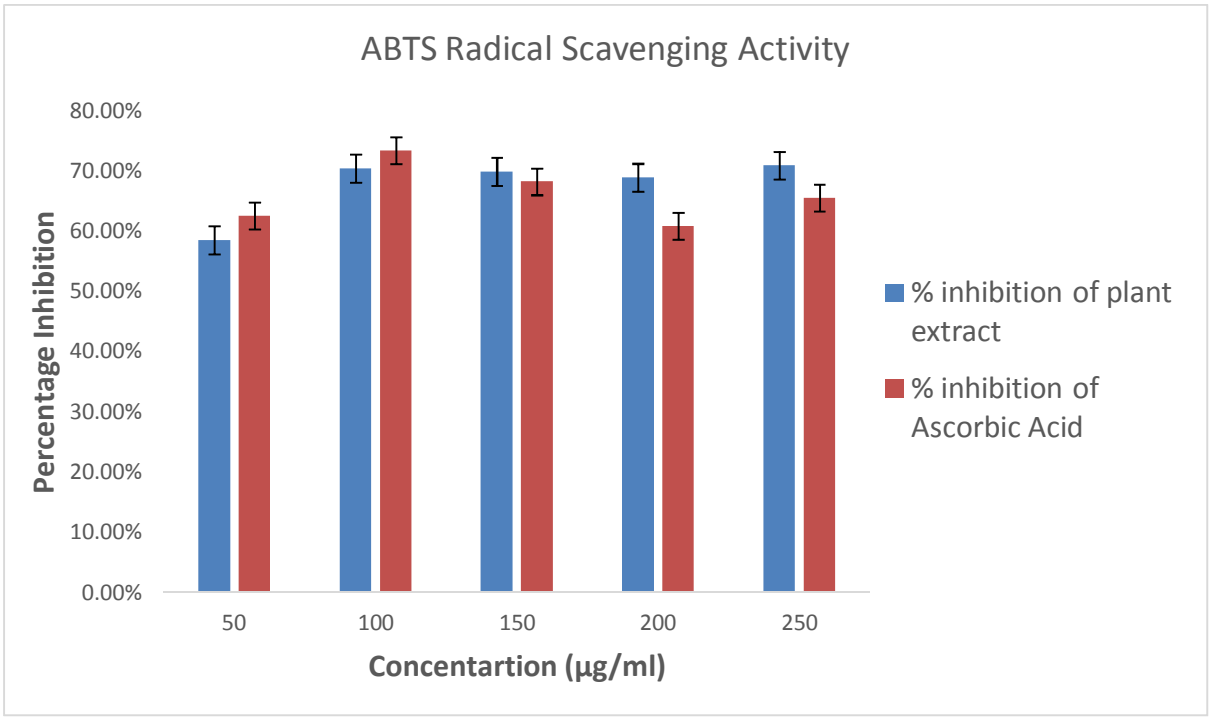


Figure 4: Percentage ABTS radical inhibition of methanolic extract of *Eucalyptus globulus.*

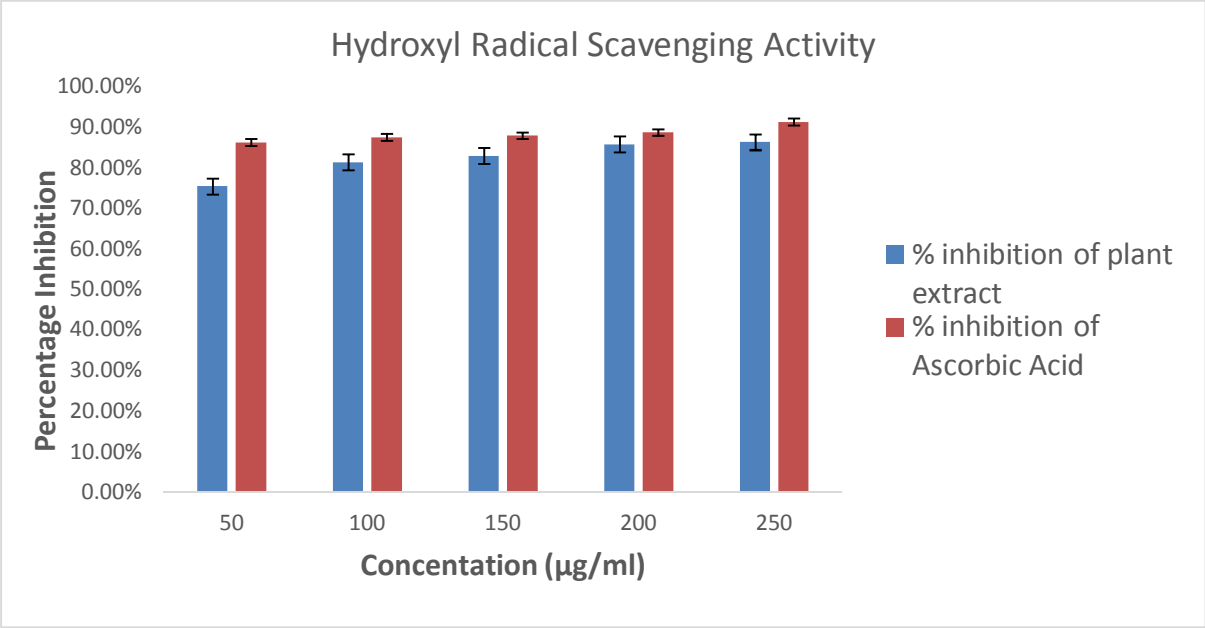


Figure 5: Percentage hydroxyl radical scavenging activity inhibition of methanolic leaf extract of Eucalyptus globulus.

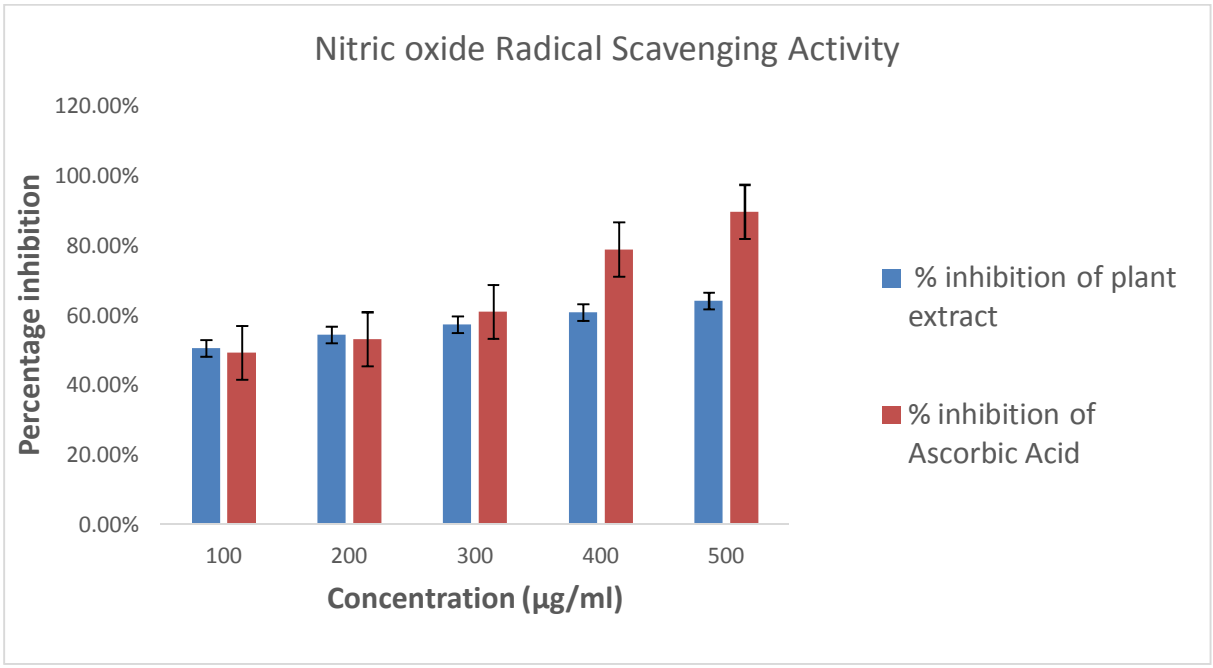


Figure 6: Percentage nitric oxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus*.

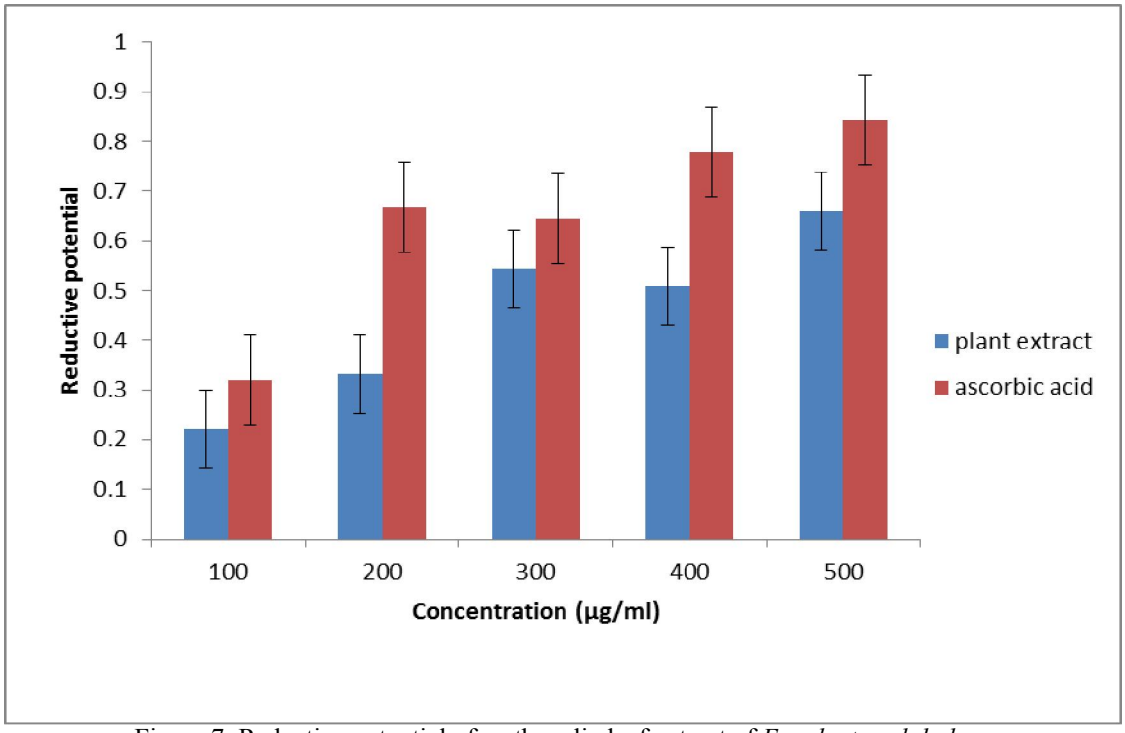


Figure.7: Reductive potential of methanolic leaf extract of *Eucalyptus globulus.*

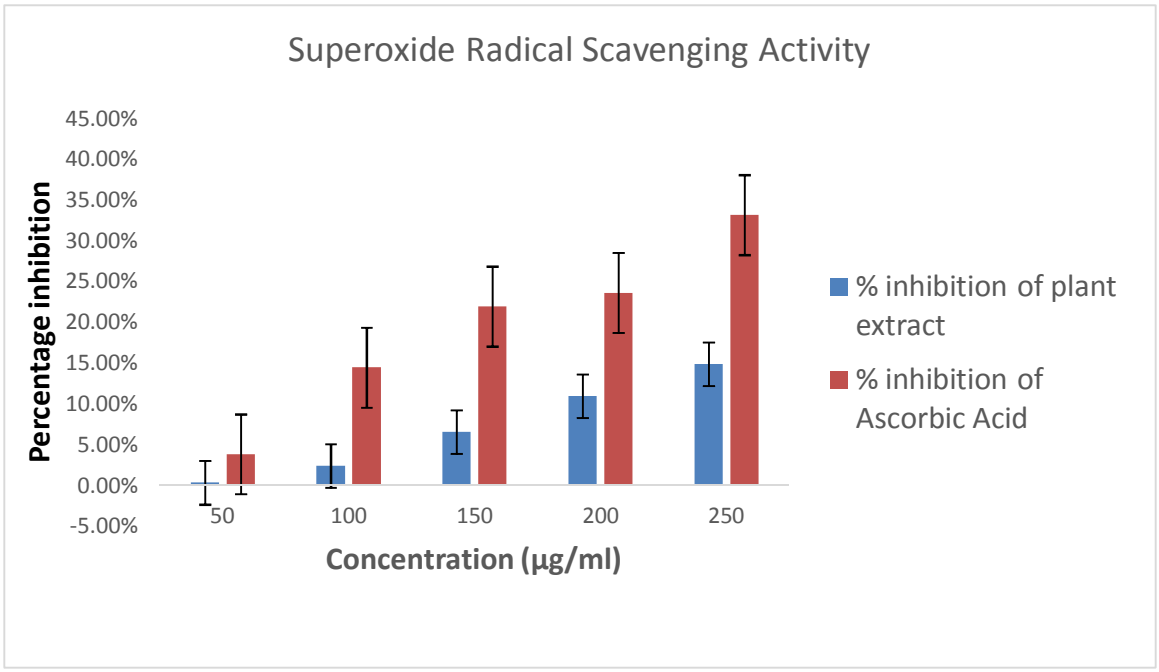


Figure. 8: Percentage superoxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus.*

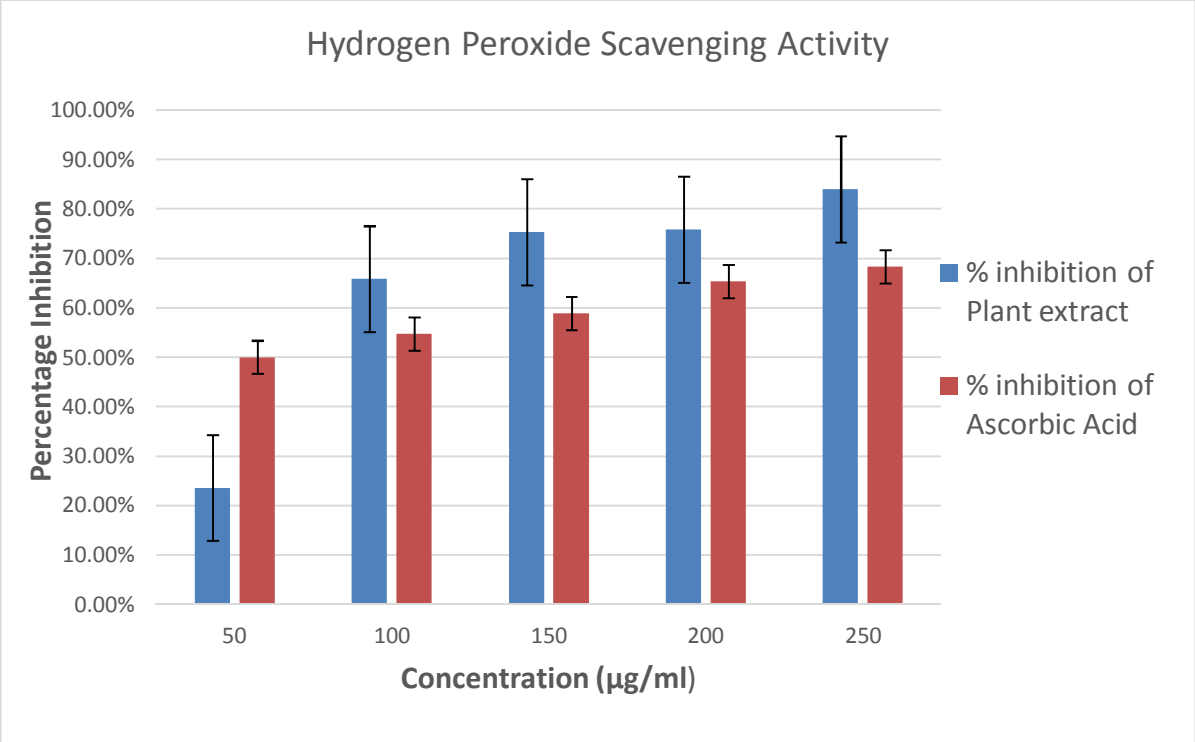


Figure.9: Percentage hydrogen peroxide radical scavenging activity inhibition of methanolic leaf extract of *Eucalyptus globulus*.

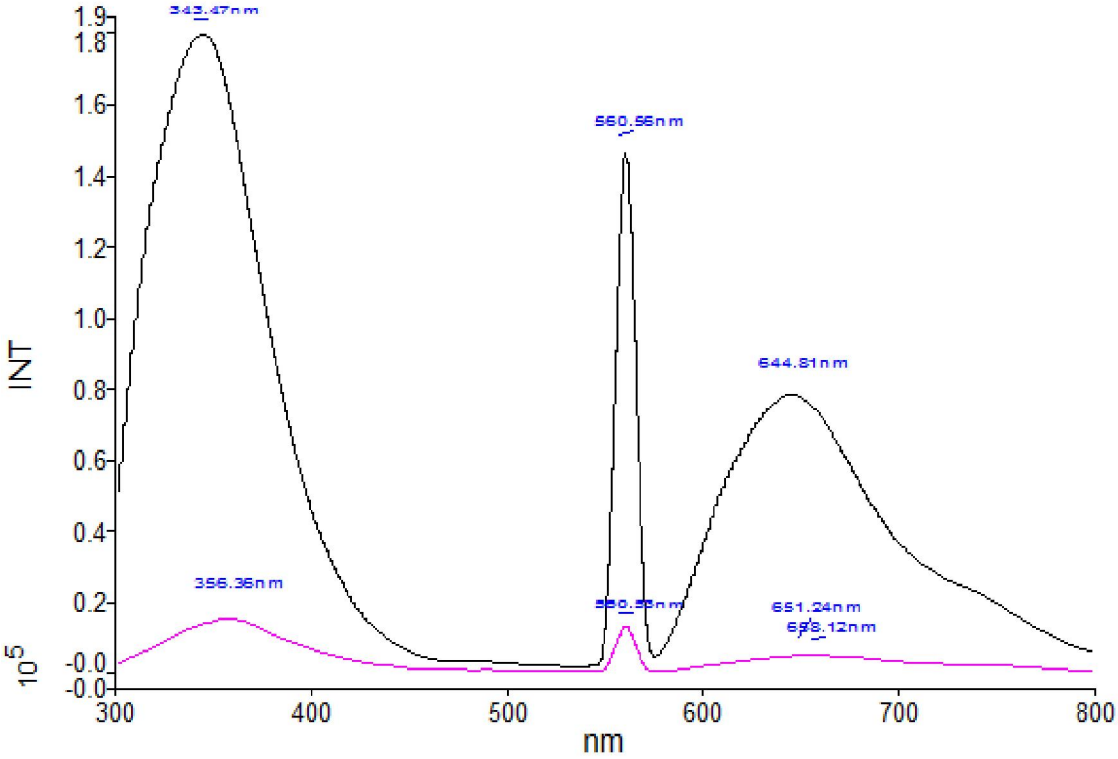
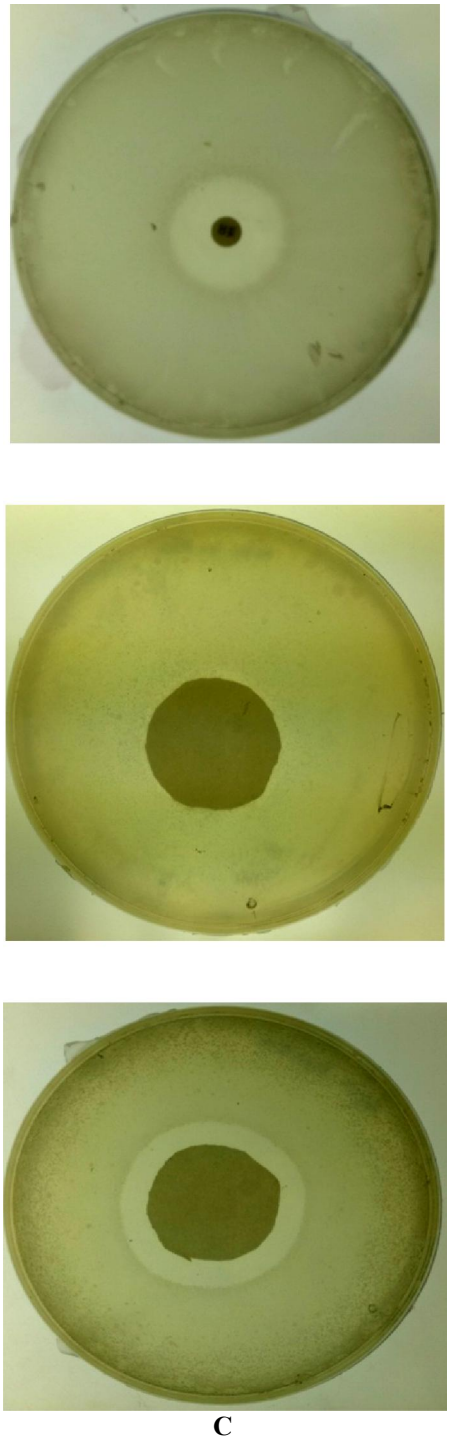


Figure.10 shows the anti-inflammatory activity of leaf extract of *Eucalyptus globulus*.



C

Figure 11. 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),

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