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Original Article

# Phyto-Analytical Studies and *in vitro* Wound Healing Activity of *Synedrella nodiflora* (L.) Gaertn Leaf Extract

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## ABSTRACT

**Background:** Traditionally among the Nigerian population *Synedrella nodiflora* is prominently used for treating Putrefied Wound. **Objectives:** The present work was designed to evaluate the *In vitro* Wound healing activity of *Synedrella nodiflora* leaf extracts. **Materials and Methods:** The current research work was aimed to perform the Qualitative phytochemical screening, FTIR, ICP-OES, Quantitative estimation of total phenolic and flavonoid content and *in vitro* pharmacological assay includes Antioxidant assay, Protein denaturation assay, cell line scratch assay for wound healing of EAE and EEs of *Synedrella nodiflora*. **Results:** Secondary phytochemicals like Flavonoid, Alkaloid, Tannin, Phenols, Terpenoids and Steroids were reported. The ICP-OES analysis concluded that the presence of elements like Zn, Fe, Cu, Mg and heavy metals like Ni, Pb, Cr and Cd were within their limits as per WHO. The EAE and EE of *Synedrella nodiflora* were assessed for their total phenolic and flavonoid content. The Antioxidant activity of EAE and EE showed $IC_{50}$ value 2.24±0.02 mg/mL and 3.72±0.02 mg/mL compared with standard Ascorbic Acid of 42.76±0.01 µg/mL. The % Inhibition of standard Diclofenac shows $IC_{50}$ value 27.99±0.026 µg/mL. whereas the $IC_{50}$ values of EAE and EE were found to be 294.98±0.02 µg/mL and 138.98±0.05 µg/mL respectively. The relative cell migration of EAE and EE were 0.041±0.05 mm and 0.036±0.01 mm at 2mcg. **Conclusion:** These studies concluded that the *Synedrella nodiflora* leaf extracts were rich in various phytochemicals and can be used for treating wounds due to its antioxidant, anti-inflammatory and wound healing potential.

**Keywords:** *Synedrella nodiflora*, Phytochemical screening, Flavonoid, Phenolics, Wound Healing.

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## INTRODUCTION

Any undesirable tissue damage resulting in the formation of wound leads to disruption of continuity towards site injury of tissues by various sets of Chemical, Physical, Immunological or Microbiological action known as biochemical reactions. Humans have localized capability of Wound healing Potential.

Plants have been considered a primary source for the treatment of various ailments since antiquity. The Secondary metabolites synthesized by plants plays a crucial role in the development of modern medicine. Globally about 80% of the population relies on classical and folk medicine for their basic needs as stated by the World Health Organization (WHO). These plant- derived medicines possess minimal or nil side effects and considered inexpensive compared to the commercial drugs available in the market.[1]

The family *Asteraceae* is a widespread plant variety which comprises about 1600 genera and 33000 species. *Synedrella nodiflora* (L). Gaertn is a small angiospermic annual weed that grows in a distinct climatic condition in various regions such as Tropical America, Andaman, West Africa, Malaysia, India, Japan, Bangladesh, China, Spain and England. The species of *Asteraceae* is rich in phytochemicals like Alkaloids, Triterpenes, Phenols, Flavonoids, Saponin, Tannins and Polyoses. The *Synedrella nodiflora* (L) Gaertn is a small, erectile annual herb. This species possesses morphological characteristics like grooved stems, dichotomous branch and swollen long internodes with hairy oppositely paired leaves up to 9 cm. The little flowers at the apex of the stem contain bunches of 2-8 efflorescence, each efflorescence with erect bracts 3-5 mm of long and floret central disc about 10-20, axillary, heterogamous. The ribbed fruits with achenes black and pappus of 2-3 bristly occurs.[2]

The species of *Synedrella nodiflora* has a history of treating various medicinal activities like anticonvulsant, anti-inflammatory, anti-malarial, anti-diarrheal, anti-bacterial, Insecticidal, analgesic Central Nervous System (CNS) depressant and anti-psychotic activity.[3-10] The current research work is proposed to study the Phyto analytical studies and *In vitro* Wound healing studies of *Synedrella nodiflora* leaf extracts.

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## MATERIALS AND METHODS

### Collection of Plant Material

*Synedrella nodiflora* species were collected from Anumandai village Villupuram district, Tamil Nadu in the month of January during Winter season. The plant was identified and authenticated by the Auroville botanical Garden, Tamil Nadu.

### Extraction

The leaves were washed by water to remove the adhered dirt and were shade dried for a period of 15 days. The dried leaves were then powdered using a mortar and pestle, passed via mesh #60 and stored in an airtight container till the study. *Synedrella nodiflora* leaf powder (30 g) was extracted by using Soxhlet extraction method for about 16 hrs with Ethyl acetate and Ethanol sequentially. The extracts were concentrated using Rotary vacuum evaporator dried extracts were kept at room temperature for further studies.[11]

### Preliminary Phytochemical Screening

The identification of Phytochemicals like terpenoids, flavonoids, alkaloids, tannins, glycosides, anthraquinones, steroids, carbohydrates and Proteins was performed using standard procedure as described in the Harborne and Biren shah with minor modifications.[12,13]

### Fourier Transform Infrared spectrophotometer (FTIR) Analysis

FTIR analysis was carried out for identification of different functional groups present in the sample Thermo Fisher (Model is 50 with In-Built ATR). A few quantities of leaf extract which was lyophilised into powdered form were encapsulated using KBr by pelletization technique. The thin film of the sample was prepared by a high pressurized system and loaded into the FTIR Spectrophotometer then the transmittance was measured between the range 400 $cm^{-1}$ to 4000 $cm^{-1}$. The samples were loaded in triplicates and bare KBr as blank. The data obtained was correlated with reference.[14,15]

### Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) Analysis

This ICP-OES analysis was carried out by using the instrument iCAP PRO thermo scientific make model with otegra version 2.18 (year 2021) software is used for detection and quantification of elements. About 1g of coarse leaf powder is weighed and undergoes wet digestion by using concentrated acids; add 5 mL of Conc.$HNO_3$, followed by 2 mL each of Con. HCl, 1 mL of 30% $H_2O_2$ into the vessel at 8000-10000 Kelvin to promote metallic solubilisation. To attain standard calibration curve serial dilutions from standard stock mix were prepared using distilled water (conductivity less than 2 µs/m) and the solution is directly injected in ICP-OES. Then linear regression curve, correlation coefficient, limits of detection and quantification were determined using obtained values.[16]

### Total Phenolic content

The total Phenolic content of EAE and EE were performed by using the Folin-Ciocalteu method. About 1 mL of sample (1 mg/mL) was added to 7.5 mL of distilled water in 10 mL volumetric flask, followed by addition of 0.5 mL FC reagent and 1 mL of 35% $Na_2CO_3$ (Sodium Carbonate) solution into it. The sample mixture was mixed well and kept aside for 30 min and the absorbance was determined at 760 nm using a double beam UV spectrophotometer [Lasany Li 2700]. To estimate the Total Phenolic content, Gallic acid standard was used as reference. The estimated values were expressed as mg of Gallic acid equivalent (GAE/g).[17,18]

### Total Flavonoid content

Total flavonoid content of *Synedrella nodiflora* leaf extracts were measured spectrophotometrically by using Aluminium chloride method as per standard procedure. About 0.5 mL concentration of the sample extract was mixed with 7.5 mL of ethanol, 0.5 mL of 1M sodium acetate, 0.5 mL of 10% aluminium chloride and 16 mL of distilled water. The solutions were maintained at room temperature for 30 min and the absorbance was measured at 415 nm using a double beam UV spectrophotometer [Lasany Li 2700]. The results were expressed as mg/g Quercetin equivalents (QE/g).[19,20]

### Antioxidant activity

The antioxidant activity of the leaf extracts of *Synedrella nodiflora* were studied using free radical scavenging efficiency by 2,2-diphenyl-1-Picrylhydrazyl (DPPH) assay method. About 1 mL various concentrations of both leaf extracts (1-5 mg/mL) were added to 6 mL methanolic solution of 0.1M DPPH. Then the mixture was shaken vigorously and kept aside for 30 min at 27°C. The absorbance was measured at 517 nm using UV-Spectrophotometer [Lasany Li 2700]. Similarly, various concentrations of Ascorbic acid (10-50 µg/mL) were used as a reference standard. The percentage of Inhibition was estimated by the given formula.[21-23]

$$\% \text{ of Free Radical inhibition} = \frac{C_A - T_A}{C_A} \times 100$$

$C_A$ - Control absorbance;

$T_A$ - Test sample absorbance

### *In vitro* Anti-inflammatory activity

*In vitro* Anti-inflammatory activity of *Synedrella nodiflora* leaf extracts were studied using egg albumin protein denaturation method. A successive dilution of leaf extracts ranging from 78.125 µg/mL to 2500 µg/mL was prepared and the reaction mixtures 0.4

mL of fresh egg's albumin and 5.6 mL of Phosphate buffer (adjust pH 6.4) were added.

Different concentrations of plant extract (4 mL) were mixed thoroughly with the above mixture. Various concentrations of reference standard ranges from 31.25 µg/mL to 1000 µg/mL was prepared that serves as Positive control. The denaturation process started when the reaction mixtures were kept in a water bath at 37°C±2°C initially for 15 min and then the temperature was maintained at 70°C±2°C for 3-5 min. The reaction mixture was cooled after the denaturation process for about 10-15 min. The absorbance was measured at 680 nm using a double beam UV-Spectrophotometer [Lasany Li 2700] before and after denaturation. The Percentage inhibition was measured by the given formula.[24-27]

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of standard}}{\text{Absorbance of control}} \times 100$$

### Cell Scratch Wound healing assay

The expansion of cell population using scratch assay was performed for the assessment of distribution and migration of 3T3-L1 cells. The cells were transferred into culture plates (6 well plates) in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum and 2% antibiotics like Streptomycin and Penicillin. Generation of the linear wound was done in the monolayer with the help of sterile 100 µL Plastic pipette tip, after the formation of a confluent cell monolayer. Phosphate buffer saline was used to remove cellular debris. Dimethyl Sulfoxide [DMSO] (<0.01%) used as solvent control and the Positive control comprises the Platelet derived growth factor (PDGF; 0.002 µg/mL). The concentration of crude extract was taken as 1 µg/mL, 2 µg/mL. The incubation of cells was performed using 5% $CO_2$ for a period of 16 hr. Microscopical images (capture pro Version 2.5 for progress® microscope camera from Jenoptik laser, Optik, system GmbH) were taken after incubation of scratched cell layer for the estimation of Relative Cell Migration.[28-32]

## RESULTS

### Phytochemical Screening

The preliminary phytochemical analysis of *Synedrella nodiflora* leaf extracts were represented in Table 1. The EAE and EEs revealed the presence of Alkaloids, Glycosides, Flavonoids, Terpenoids, Tannins, Phenols, Steroids and Reducing sugars.

### FTIR analysis

The FTIR analysis elucidates the functional group of unknown composition of secondary metabolites that may be present in the EAE and EE which were represented in Figures 1 and 2. The spectral peak value ranges from 4000 $cm^{-1}$ to 1500 $cm^{-1}$ usually considered as functional group region and the range below 1500 $cm^{-1}$ to 600 $cm^{-1}$ may be considered as fingerprint regions for identification of traces element, alkyl or aryl bond. The presence of compounds like ester (1736.60 $cm^{-1}$), phenol (1384.56 $cm^{-1}$), alkane methylene group (1462.94 $cm^{-1}$) and aldehyde (1381.81 $cm^{-1}$) spectral ranges may indicate the presence of aromatic compound like terpenoid class. The compound class of functional groups like delta lactone or 6 membered lactone group (1737.48 $cm^{-1}$), sulphonamide (1164.96 $cm^{-1}$) and intermolecular bonded alcohol (3402.02 $cm^{-1}$) may be representing the presence of cardiac glycoside. The existence of primary amine (3406.67 $cm^{-1}$), phenols (1384.56 $cm^{-1}$), ester group may elucidate the presence of flavonoids. The presence of conjugated alkene (1630.36 $cm^{-1}$), carboxylic acid dimer (1710.32 $cm^{-1}$), secondary alcohol (1113.82 $cm^{-1}$), aldehyde (1737.48 $cm^{-1}$), ketone (1710.32 $cm^{-1}$) may illustrate the presence of tannins. Hence the EAE and EE may possess certain classes of secondary metabolites like terpenoid, tannin, flavonoids and glycosides as reported in Tables 2 and 3 respectively.

### ICP-OES Analysis

The ICP-OES analysis result showed that the presence of some Inorganic trace elements in various concentration were Magnesium (2060.20ppm), Zinc (46.97ppm), Chromium (45.59ppm), Copper (33.08ppm), Titanium (21.91ppm), Vanadium (3.82ppm), Nickel (3.80ppm), Lead (3.55ppm) and Cadmium (0.047ppm) respectively. The Quantitative elemental detection of 11 elements was represented in Figure 3.

### Total Phenolic Content

The Total Phenolic Content of EAE and EE were represented in Table 4. The Quantitative estimation of *Synedrella nodiflora* leaf extracts by using Folin-Ciocalteu Reagent is stated in terms of Gallic acid equivalent and they were expressed as mg GAE/mg. The Linear Regression Curve of Gallic acid Standard was represented in Figure 4. The EAE showed higher concentration of Phenol as compared to EE.

The data obtained were expressed as Mean±Standard Deviation ($n$=3).

### Total Flavonoid content

The result for Total Flavonoid content of EAE and EE were represented in Table 5. The Linear Regression Curve of Quercetin Standard was represented in Figure 5. The Flavonoid quantity was estimated spectrophotometrically by the Aluminium Chloride Colorimetric method and was expressed in the terms of Quercetin Equivalent (QE/mg). The EAE exhibited higher flavonoid content as compared to EE.

### Antioxidant activity

The extracts were tested for their free radical scavenging capacity by the DPPH assay method using ascorbic acid as a standard. The degree of scavenging activity was summarized in Table 6 and the

**Table 1:** **Result of Phytochemical analysis of *Synedrella nodiflora* leaf extracts.**

| Sample | Flavonoid | Alkaloid | Phenol | Glycoside | Steroid | Tannin | Terpenoid | Saponin | Reducing sugar |
|---|---|---|---|---|---|---|---|---|---|
| EAE | + + + | + | + + + | + + | + + | + + | + + + | - | + + |
| EE | + + | + | + + | + + | + | + + | + + + | - | + + |

EAE- Ethyl acetate extract; EE- Ethanolic extract. +: positive; -: negative.

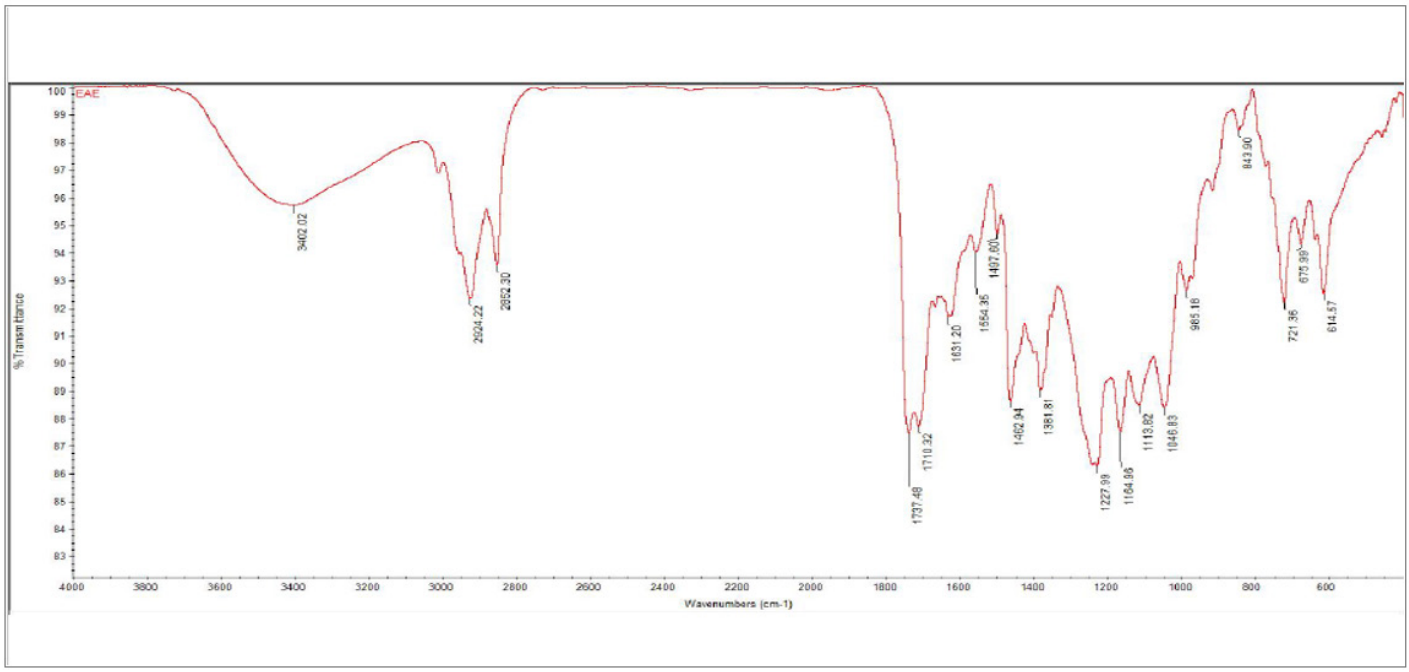


**Figure 1:** FTIR spectra of EAE of *Synedrella nodiflora*.

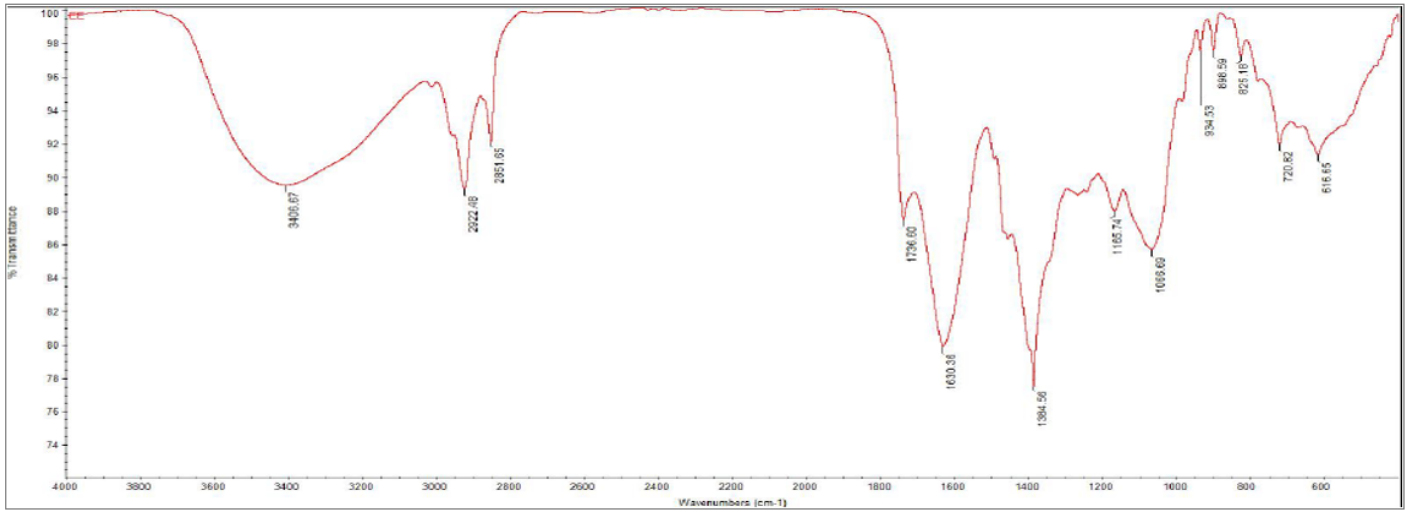


**Figure 2:** FTIR spectra of EE of *Synedrella nodiflora*.

percentage of inhibition was represented in Figures 6-8. The $IC_{50}$ values were obtained using the Linear regression curve method and shown in Table 6.

## Protein denaturation assay

The *In vitro* anti-inflammatory activity for EAE and EE of *Synedrella nodiflora* was determined by using egg albumin denaturation assay using double beam UV-spectrophotometer [Lasany Li 2700] and the results were expressed in the Tables 7 and 8 which were Interpreted from Figures 9-11. This study proves that the percentage inhibition of protein denaturation depends upon concentration. However, the EAE shows more percentage inhibition than the standard Diclofenac, but EE shows less percentage inhibition compared with standard. The

**Table 2: Result of FTIR analysis on EAE of *Synedrella nodiflora*.**

| Sl. No. | Peak frequency $cm^{-1}$ | Reference Frequency Range $cm^{-1}$ | Functional group | Intensity of functional group | Compound class |
|---|---|---|---|---|---|
| 1. | 3402.02 | 3550-3400 | N-H stretching | Medium | Primary amine |
| 2. | 2924.22 | 3300-2500<br>3000-2840 | O-H stretching<br>C-H stretching | Strong, Broad<br>Medium | Carboxylic acid<br>Alkane |
| 3. | 2852.30 | 3000-2840 | N-H stretching | Strong, Broad | Amine salt |
| 4. | 1737.48 | 1750-1735 | C=O stretching | Strong | Ester (6-membered Lactone) |
| 5. | 1710.32 | 1720-1705 | C=O stretching | Strong | Carboxylic acid olimer |
| 6. | 1631.20 | 1650-1600 | C=C stretching | Medium | Conjugated alkene |
| 7. | 1554.35 | 1550-1500 | N-O stretching | Strong | Nitro compounds |
| 8. | 1497.60 | 1550-1500 | N-O stretching | Strong | Nitro compounds |
| 9. | 1462.94 | 1465 | C-H bending | Medium | Alkane Methylene group |
| 10. | 1381.81 | 1390-1380<br>1390-1310<br>1385-1380 | C-H bending<br>O-H bending<br>C-H bending | Medium<br>Medium<br>Medium | Aldehyde<br>Phenol<br>Alkane gem dimethyl |
| 11. | 1275 | 1275-1200 | C-O stretching | Strong | Alkyl aryl ether |
| 12. | 1164.96 | 1170-1155 | S=O stretching | Strong | Sulfonamide |
| 13. | 1113.82 | 1124-1087 | C-O stretching | Strong | Secondary alcohol or Aliphatic ether |
| 14. | 1046.83 | 1050-1040 | CO-O-CO stretching | Strong, Broad | Anhydride |
| 15. | 985.18 | 995-985 | C=C bending | Strong | Alkene monosubstituted |
| 16. | 843.90 | 850-550 | C-Cl stretching | Strong | Halo compound |
| 17. | 721.36 | 700±20 | C-H bending | Strong | monosubstituted benzene derivative or Tri substituted |
| 18. | 675.99 | 730-665 | C=C bending | Strong | Alkene Disubstituted (cis) |
| 19. | 614.57 | 690-515 | C-Br stretching | Strong | Halo compound |

$IC_{50}$ values of EAE, EE and standard Diclofenac were found to be 138.98±0.05 µg/mL, 294.98±0.02 µg/mL and 27.99±0.026 µg/mL respectively.

### Cell scratch assay method

The relative fibroblast cell migration of EAE and EE of *Synedrella nodiflora* were represented in Figure 12 and found to be 0.036 mm, 0.029 mm and 0.036 mm, 0.041 mm at lower and higher concentration respectively. The relative cell migration of standard (PDGF) was found to be 0.042mm. Those results were represented in Table 9 which showed that EAE exhibited increased Wound healing activity compared to the EE.

## DISCUSSION

Evaluation of the biologically compounds present in plant extracts, present in predominantly by phytochemical screening may be helpful in the identification of therapeutically effective plant sources.[33] Terpenoids are reported to possess different pharmacological activities including anti-inflammatory, anti-malarial, anti-cancer, anti-microbial and hypoglycemic activity.[34] Flavonoids exhibit wide range of biological activity as

**Table 3: Result of FTIR analysis on EE of *Synedrella nodiflora*.**

| Sl. No. | Peak frequency $cm^{-1}$ | Reference Frequency Range $cm^{-1}$ | Functional group | Intensity of functional group | Compound class |
|---|---|---|---|---|---|
| 1. | 3406.67 | 3550-3400 | N-H stretching | Medium | Primary amine |
| 2. | 2922.48 | 3300-2500 | O-H stretching | Strong, Broad | Carboxylic acid |
| 3. | 2851.65 | 3000-2840 | C-H stretching | Medium | Alkane |
| 4. | 1736.60 | 1750-1735 | C=O stretching | Strong | Ester (6-membered Lactone) |
| 5. | 1630.36 | 1650-1600 | C=C stretching | Medium | Conjugated alkene |
| 6. | 1384.56 | 1390-1380<br>1390-1310 | C-H bending<br>O-H bending | Medium<br>Medium | Aldehyde<br>Phenol |
| 7. | 1165.74 | 1165-1150 | S=O stretching | Strong | Sulfonic acid |
| 8. | 1066.69 | 1085-1050 | C-O stretching | Strong | Primary alcohol |
| 9. | 934.53 | 915-905 | C=C bending | Strong | Alkene monosubstituted |
| 10. | 898.59 | 880±20 | C-H stretching | Strong | 1,2,4-Tri substituted |
| 11. | 825.18 | 810±20 | C-H bending | Strong | 1,2,3,4-Tetra substituted, or 1,4-Di substituted |
| 12. | 720.82 | 700±20 | C-H bending | Strong | 1,3-Disubstituted or monosubstituted benzene derivative |
| 13. | 616.65 | 690-515 | C-Br stretching | Strong | Halo compound |

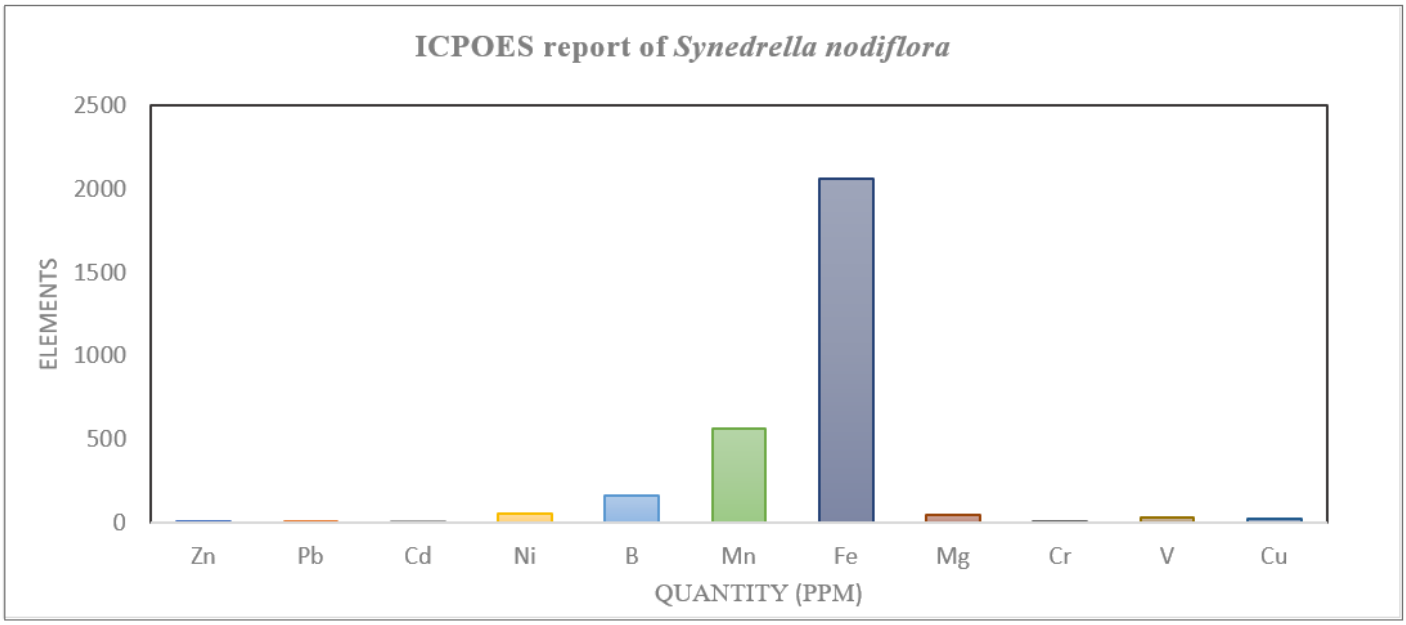


**Figure 3:** ICP-OES analysis of *Synedrella nodiflora* coarse leaf Powder.

anti-oxidant, anti-diabetic, anti-microbial, anti-inflammatory.[35] Additionally, flavonoids were also recognized for its wound healing property.[36] Alkaloids are well known for its anti-inflammatory, analgesic, anti-cancer activity and also for promoting wound healing.[37] The phenolic compounds and tannins were reported to possess anti-diabetic, anti-oxidant and antibacterial activity.[38,39] In the current study, FTIR spectroscopy is used for identifying different functional groups present in the leaf extracts of *Synedrella nodiflora* based on the measurement of the peak values.[40] The FTIR results of *Synedrella nodiflora* leaf extract

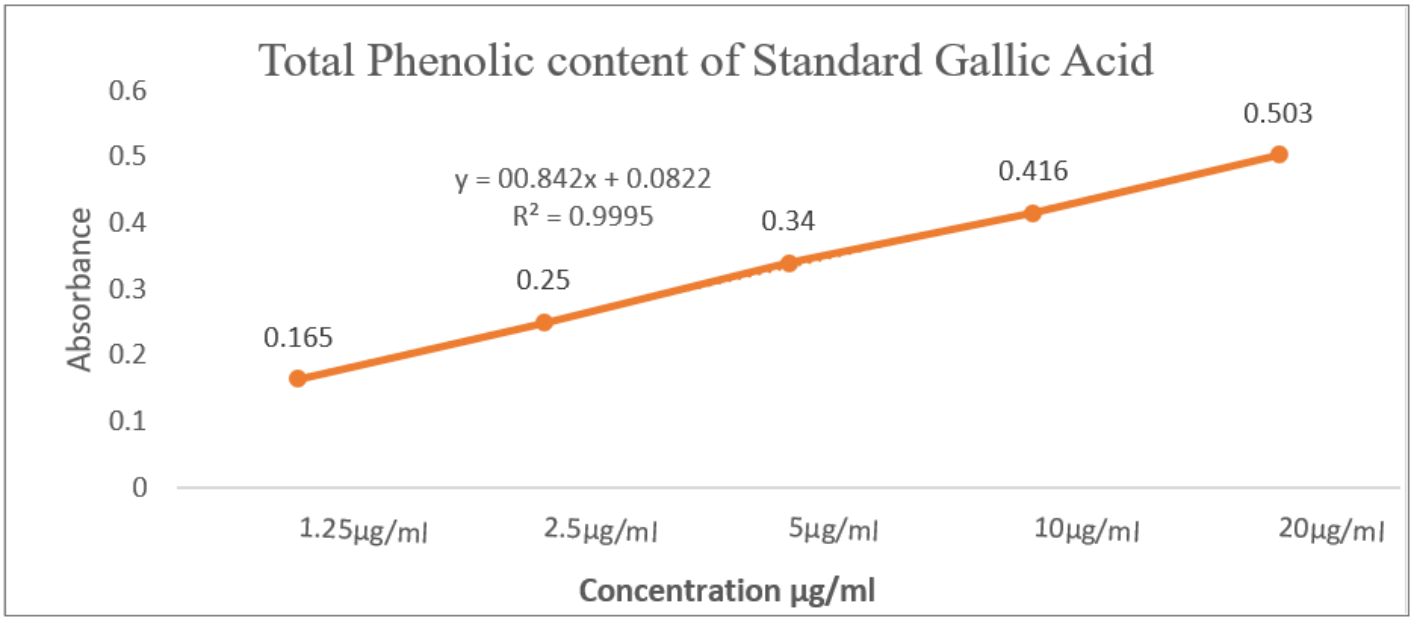


**Figure 4:** Linear Regression Curve of Gallic acid Standard.

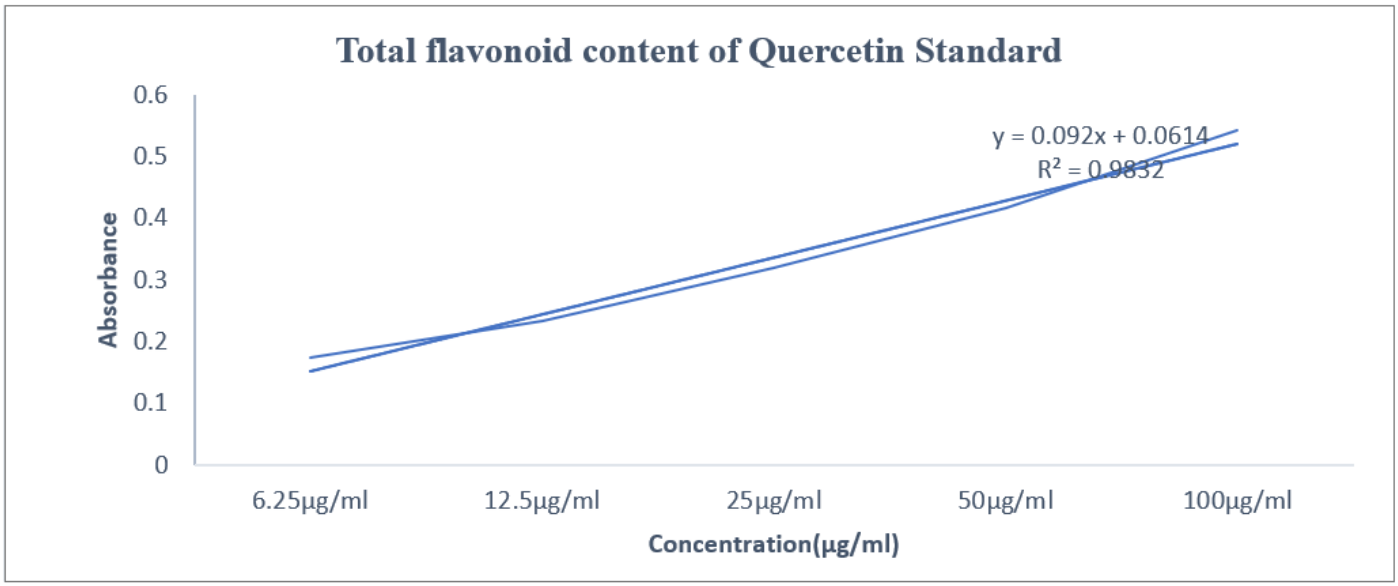


**Figure 5:** Linear Regression Curve of Quercetin Standard.

reported the presence of groups indicating the phytochemicals like terpenoid, tannin, flavonoids and glycosides previously reported to contribute the anti-bacterial, wound healing activity. ICP-OES is a widely used method for element analysis due to its ability as a multiple wavelength detector for identification and quantification of elements. Among various traditional elemental analysis methods like spectroscopic, volumetric, gravimetric and colorimetric, the inductively coupled plasma methods like mass spectrometry (ICP-MS) and optical emission spectroscopy (ICP-OES) indicates higher accuracy and precision rate. Under the controlled condition ICP-OES exhibits spectral plasma excitation at different wavelengths for each distinct element. The emission of light intensity is compared with concentration of each element.[41] Magnesium plays a vital role in initiating

**Table 4: Total Phenolic Content of *Synedrella nodiflora* leaf extract.**

| Sample | Gallic acid equivalent (GAE/mg) |
|---|---|
| EAE | 0.0040±0.0003 |
| EE | 0.0025±0.0001 |

wound healing through different metabolic pathways and cellular mechanism. It contributes nearly 3000 enzymes for ATP, RNA and DNA formation.[42] Iron, an important element of hemoglobin and myoglobin. It catalyzes generated free radicals and promotes tissue injury and cellular death. According to the recommended dietary allowance, normal intake of iron for women is 18 mg/day and for men 10 mg/day. Zinc, an integral part of proteins which

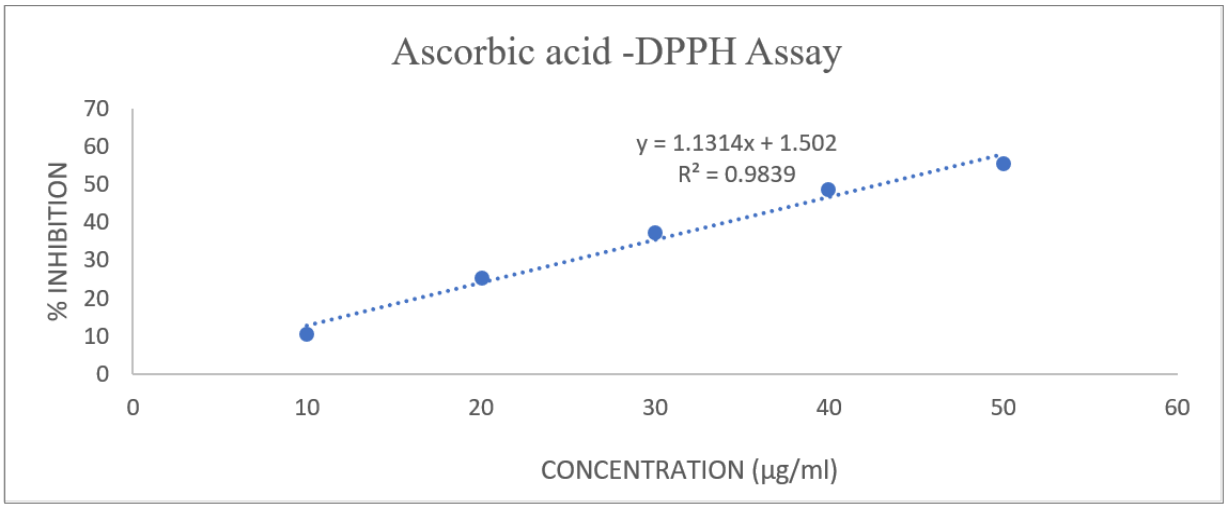


**Figure 6:** DPPH assay of Ascorbic acid using Linear Regression method.

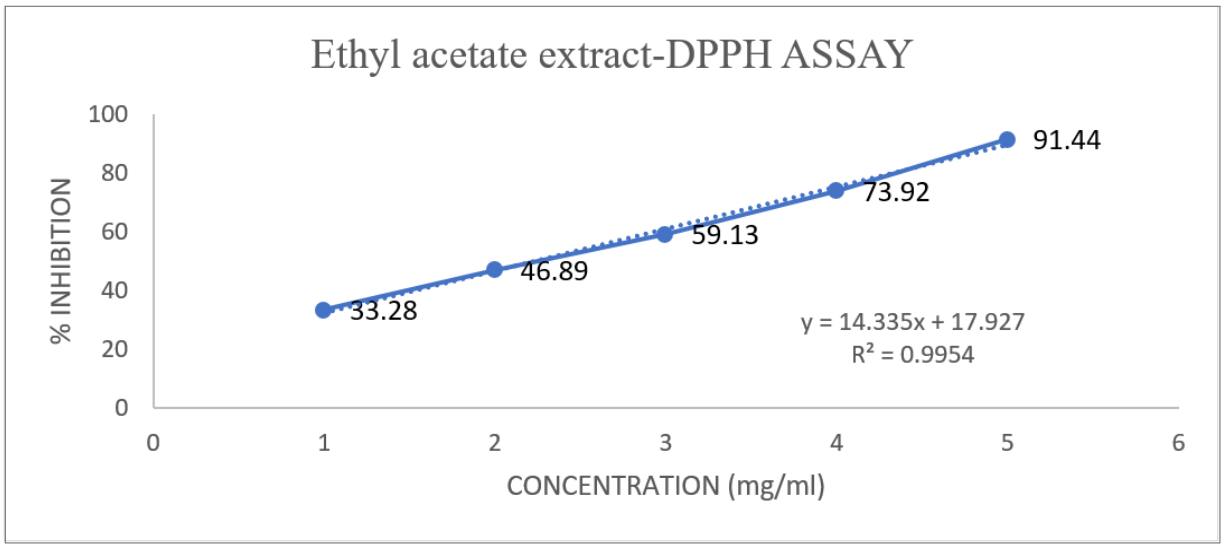


**Figure 7:** DPPH assay of EAE of *Synedrella nodiflora* using Linear Regression method.

**Table 5:** **Total flavonoid Content of *Synedrella nodiflora* leaf extract.**

| Sample | Quercetin equivalent (QE/mg) |
|---|---|
| EAE | 0.051±0.004 |
| EE | 0.020±0.0025 |

The obtained results were expressed as Mean±Standard Deviation (n=3).

**Table 6:** **$IC_{50}$ values of *Synedrella nodiflora* leaf extract using DPPH assay.**

| Sample | $IC_{50}$ Value |
|---|---|
| Ascorbic acid | 42.76±0.01 µg/mL |
| EAE | 2.24 ±0.02 mg/mL |
| EE | 3.72 ±0.02 mg/mL |

The obtained results were performed in triplicate using Mean±Standard Deviation (*n*=3).

stabilize cellular membrane, cell respiration, wound healing, synthesis of DNA and protein and regulator homeostasis. The recommended normal level for zinc was 15 mg per day for an adult.[43] Copper is an essential element that plays a complex role in the mechanism of wound healing and cytokines modulators. Which tolerate the expression of collagen and fibrinogen formation The average intake of copper varies among 28 countries for adult ranging 1.6 to 3.3 mg/day.[44] Manganese and Zinc combination used for wound healing by changing integrin expression and the average intake of manganese for adult was 2.5 to 5 mg/day.[45] Boron is used as diet supplements because it has interaction with magnesium, Vitamin D and Calcium. It also contributes to anti-oxidant and anti-inflammatory activity.[46] The heavy metal like lead permissible level for human adult intake was 7 µg/kg body mass/day, according to WHO guidelines. Cadmium intake should not exceed 0.05 mg/kg according to the United States FDA from NCBI guidelines. Chromium, an essential element for carbohydrate metabolism but it comes under grade2 heavy metal. The average intake of humans differs in different regions. so, the National Research Council (1980) set the limit 200 µg/day for adults. All the elements described above were within their limit as said by WHO guidelines, FDA, NCBI

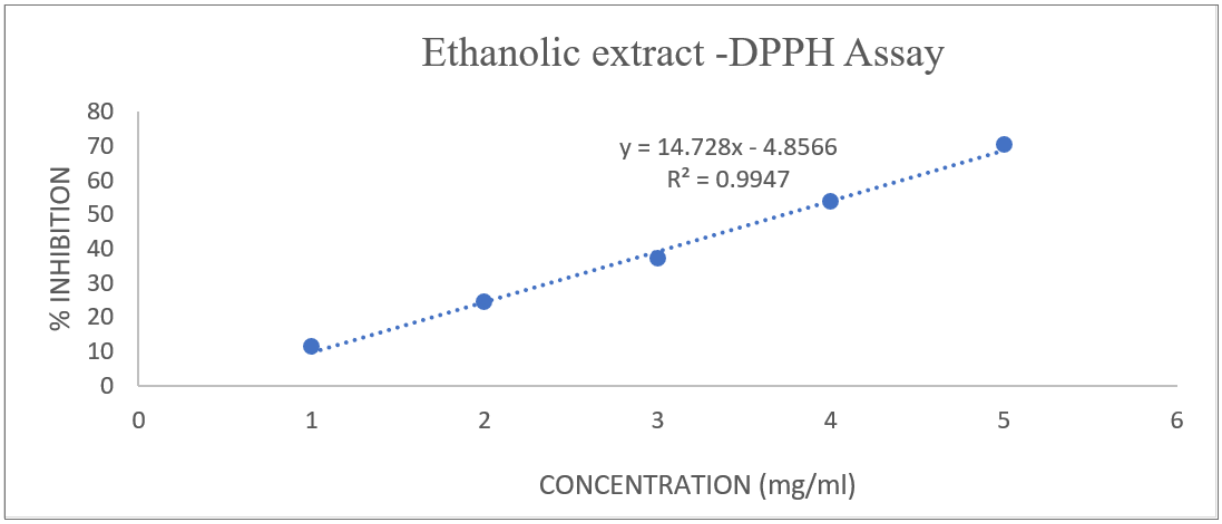


**Figure 8:** DPPH assay of EE of *Synedrella nodiflora* using Linear Regression method.

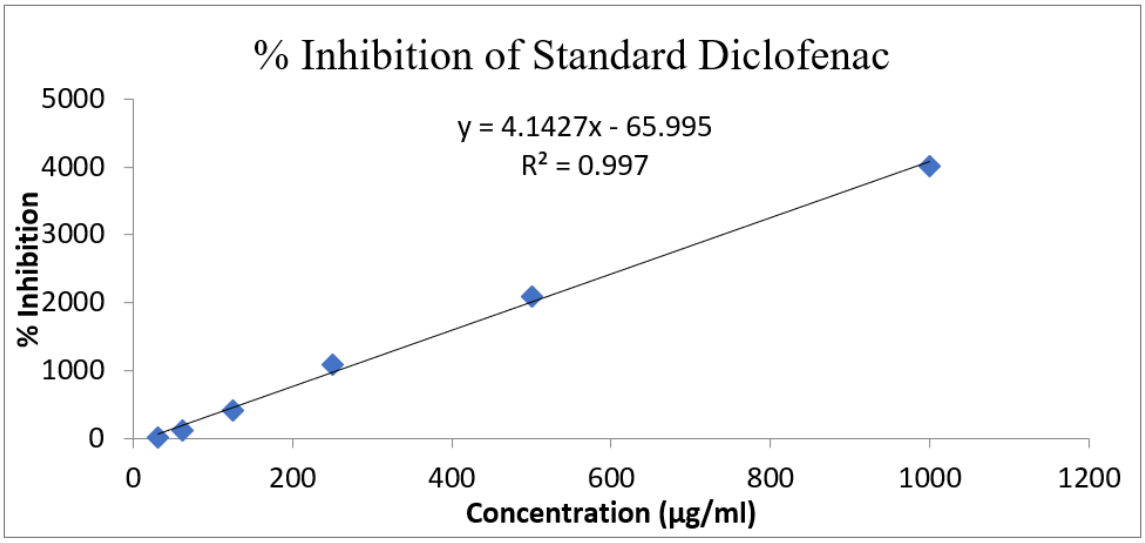


**Figure 9:** Protein Denaturation assay of Standard Diclofenac using Linear Regression method.

**Table 7:** **The Percentage inhibition rate of Standard Diclofenac.**

| Concentration (µg/mL) | % Inhibition |
|---|---|
| 31.25 | 25±0.01 |
| 62.5 | 125±0.05 |
| 125 | 410±0.03 |
| 250 | 1090±0.03 |
| 500 | 2100±0.04 |
| 1000 | 4010±0.02 |

Each value expresses the Mean±Standard deviation (*n*=3).

**Table 8:** **The Percentage inhibition rate of *Synedrella nodiflora* leaf extract.**

| Concentration (µg/mL) | %Inhibition of EAE | % Inhibition of EE |
|---|---|---|
| 78.125 | 20±0.001 | 12.5±0.001 |
| 156.5 | 55±0.001 | 25±0.003 |
| 312.5 | 122.6±0.005 | 50±0.0004 |
| 625 | 248.7±0.35 | 120±0.005 |
| 1250 | 505.9±0.01 | 210.5±0.1 |
| 2500 | 1000.6±0.2 | 456.6±0.5 |

Data were obtained from three independent experiments, each performed in triplicates and represented as Mean±SD.

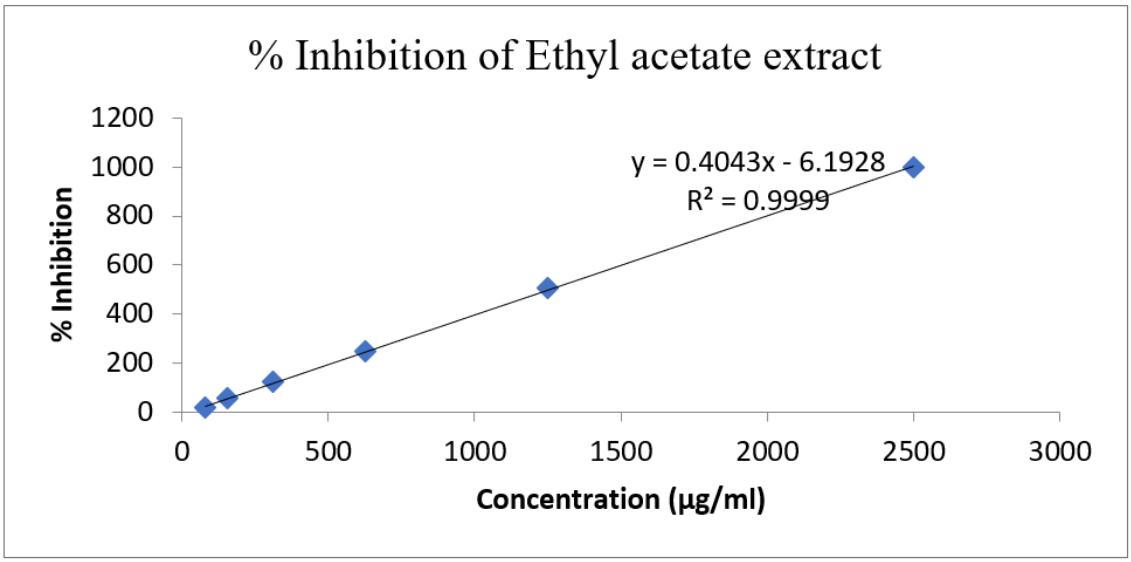


**Figure 10:** Protein Denaturation assay of EAE of *Synedrella nodiflora* using Linear Regression method.

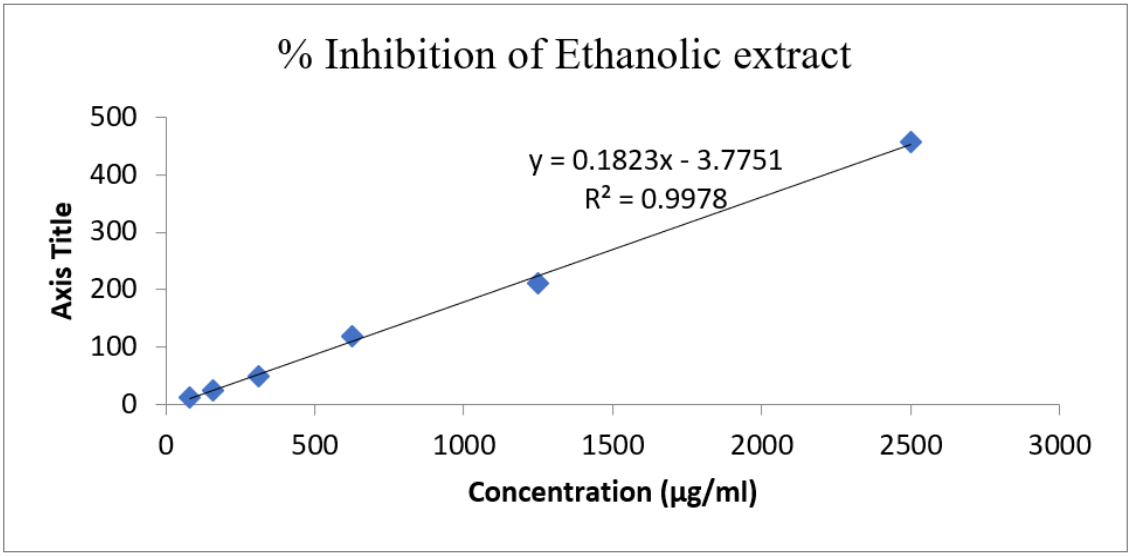


**Figure 11:** Protein Denaturation assay of EE of *Synedrella nodiflora* using Linear Regression method.

guidelines and ICH guidelines. Plant phenolic compounds are considered as primary antioxidants or free scavengers and play a major role associated with the treatment of various diseases. FC reagent method is the most employed for the quantification of phenolic compounds. The extracts containing phenolic compounds react with FC reagent in an alkaline medium forming blue coloured Phosphomolybdic/Phosphotungstic acid complex.[47] The antioxidant activity produced by flavonoids involves inhibiting the formation of reactive oxygen and chelating the trace elements involved in the production of free radicals. The radical scavenging activity of flavonoids were reported on free radicals and oxidizing molecules and the potency depends upon the presence of OH groups. The antioxidant activity produced by flavonoids involves inhibiting the formation of reactive oxygen and chelating the trace elements involved in the production of free radicals.[48] DPPH assay method is used to measure the electron donating capacity of natural compounds based on the free radical scavenging property. The antioxidant activity of the compound is proportional to the degree of decolourization of the DPPH solution. A significant reduction in the absorbance of the reaction mixture indicates the higher inhibition percentage of the compound under study. In the current study the Ethyl acetate and EEs showed higher inhibition percentage may be due to the higher phenolic content.[49] The main mechanisms involved in protein denaturation are modification of hydrogen atoms involved in electrostatic interaction, hydrophobic interaction and disulfide linkages. The production of auto antigens by denaturation is caused due to inflammations in clinical conditions like rheumatoid arthritis, cancer, rheumatoid arthritis and diabetes. The anti-inflammatory activity of a drug can be measured by its ability to inhibit denaturation of protein. Both the extracts were found to inhibit the protein denaturation

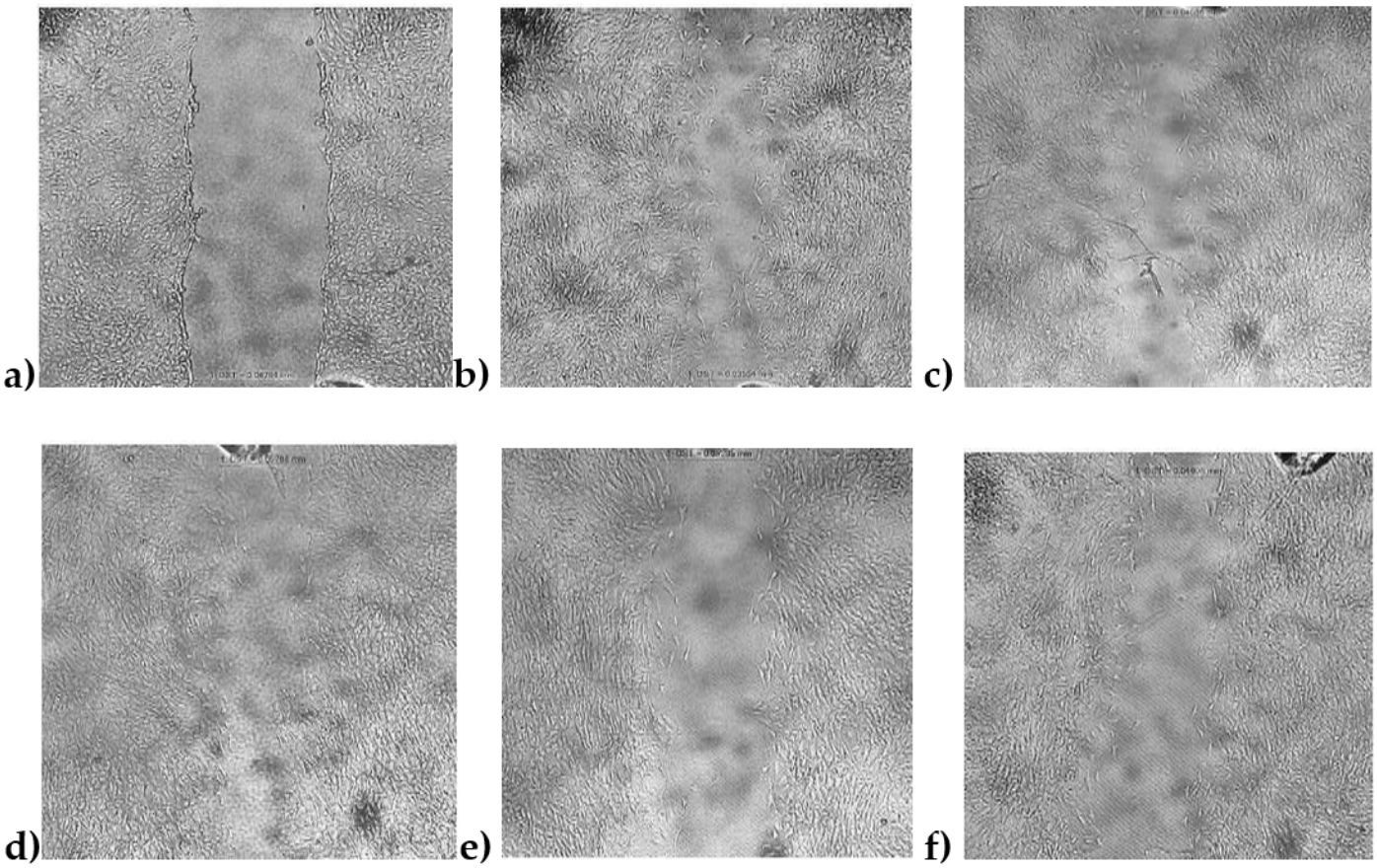


**Figure 12:** Microscopical images of *in vitro* scratch assay results of *Synedrella nodiflora* leaf extracts showing fibroblast cell migration after 24 hr of induction of Wound in the following groups a) DMSO; b) PDGF; c) EAE 1-mcg; d) EAE 2-mcg; e) EE 1-mcg; f) EE 2-mcg.

**Table 9: The Relative cell migration rate of *Synedrella nodiflora* leaf extracts.**

| Sl. No. | Treatment Group | Concentration (µg/mL) | Relative Cell Migration (mm) at 24hr. |
|---|---|---|---|
| 1. | 1% DMSO | | 0.032±0.001 |
| 2. | PGDF | 0.002 | 0.042±0.003 |
| 3. | EAE | 1 | 0.036±0.0005 |
| | | 2 | 0.041±0.0005 |
| 4. | EE | 1 | 0.029±0.001 |
| | | 2 | 0.036±0.001 |

Experimental groups were performed in triplicates and interpolated by using Mean±Standard Deviation ($n$=3).

by reducing the synthesis of collagen, mucopolysaccharide and reducing the number of fibroblasts.[50] DMEM is the Dulbecco's modified eagle medium that is mainly used as growth stimulators in various mammalian cells. The role of DMEM in Scratch assay is regeneration of fibroblast and re-epithelization involved in the secondary phase of wound healing using Fetal bovine serum as supplement and Platelet derived growth factor (PDGF) as growth regulator during cell division. PDGF is used as positive control due to peptide matrix production, synthesis of many epithelial and connective tissues thereby increasing the efficiency of wound healing. To elucidate the effectiveness of wound healing, the wound closure time is frequently observed with a specific range of time intervals that determines cell proliferation. Some of the studies showed that above 0.5%DMSO concentration produces toxic effects, our study has 0.01% which is negligible. For better understanding, comparative study between EAE and EE of *Synedrella nodiflora* at different concentrations with specific time intervals for cell migration and proliferation were studied with 0.02 µg/mL of standard PDGF. The EAE shows increased relative cell migration compared to EE within 0-24 hr simultaneously. The results compared with positive control confirmed that EAE had relatively the same proliferation activity.[51]

## CONCLUSION

This is the first study reporting Wound healing potency of *Synedrella nodiflora*. The therapeutically important phytochemicals and elements like Magnesium, Iron, Zinc and Copper were reported in *Synedrella nodiflora*. All these constituents may contribute to the Wound healing activity of *Synedrella nodiflora* leaf extract. This study scientifically supported the traditional claim of *Synedrella nodiflora* for the treatment of wounds.

In the current study, the results scientifically support the traditional claim of wound healing property of *Synedrella nodiflora* plant. The results of anti-inflammatory activity of the plant extract demonstrated its potential to support wound healing. The presence of phenolic compounds, flavonoids, alkaloids, terpenes and tannins also plays a significant role in wound healing. The presence of elements like Copper, Magnesium and Zinc also plays a major role in initiating the Wound healing process. Further studies on isolating the phytochemical from *Synedrella nodiflora* will be beneficial in revealing the wound healing potential to limelight.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**EAE:** Ethyl acetate extract; **EE:** Ethanol extract; **WHO:** World Health Organization; **CNS:** Central Nervous System; **FTIR:** Fourier transform infrared spectrophotometer; **ICP-OES:** Inductively Coupled Plasma-Optical Emission Spectrometry; **FC:** Folin Ciocalteu; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **DMEM:** Dulbecco's modified Eagle Medium; **DMSO:** Dimethyl Sulfoxide; **EAE:** EAE; **EE:** EE; **FDA:** Food and drug administration; **ICH:** International council for Harmonisation; **NCBI:** National center for Biotechnology Information; **PDGF:** Platelet-derived growth factor.

## SUMMARY

Development of wounds has become one of the serious concerns especially among the patients with diabetics. Phytochemical studies on *Synedrella nodiflora* leaf extract reported the presence of flavonoid, phenol, terpenoids, tannins and steroids. These phytochemicals are reported to possess Wound healing activity. The elemental analysis of *Synedrella nodiflora* leaf extract revealed the presence of the elements like Magnesium, Iron, Zinc and Copper that have a vital role in promoting Wound healing. Magnesium initiates Wound healing while Iron promotes Wound healing by avoiding damage to the tissues and cell line death. Zinc is also an important element for promoting Wound healing by stabilizing cellular membranes and regulating Hemostasis. Copper is an essential element to produce collagen and fibrinogen there by promoting Wound healing. Terpenoids also accelerate Wound healing by promoting the production of collagen thereby promoting the Healing process. GCMS reports of *Synedrella nodiflora* leaf extracts reported the presence of high amounts of Phytol, a diterpene alcohol. Phytol reduces inflammation by inhibiting nuclear factor-Kappa B (NF-κB Pathway). It also provides better remodeling of tissue during Wound healing.

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