INTRODUCTION

Plants, which have one or more of its parts having substances that can be used for treatment of diseases, are called medicinal plants\(^1\). Medicines derived from plants are widely famous due to their safety, easy availability and low cost\(^2\). Throughout the ages, humans have relied on nature for their basic needs, for the production of food, shelter, clothing, transportation, fertilizers, flavours, fragrances, and medicines\(^3\). Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use. The first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2 600 BC\(^4\). Among the substances that were used are oils of Cedrus species (cedar) and Cupressus sempervirens (cypress), Glycyrrhiza glabra (licorice), Commiphora species (myrrh) and Papaver somniferum (poppy juice), all of which are still in use today for the treatment of ailments ranging from coughs and colds to parasitic infections and inflammation. In ancient Egypt, bishop’s weed (Ammimajus) was reported to be used to treat vitiligo, a skin condition characterized by a loss of pigmentation\(^5,6\). More recently, a drug (methoxypsoralen) has been produced from this plant to treat psoriasis and other skin disorders, as well as T-cell lymphoma\(^8\). The interest in nature as a source of potential chemotherapeutic agents continues. Natural products

ABSTRACT

Objective: Cocos nucifera (L.) (Arecaecae) is commonly called the “coconut tree” and is the most naturally widespread fruit plant on Earth. Throughout history, humans have used medicinal plants therapeutically, and minerals, plants, and animals have traditionally been the main sources of drugs. The objective in the present study was to screen the phytochemical profile and pharmacological activities of methanolic extract of Cocos nucifera (L.) leaves.

Methods: To investigate pharmacological activities DPPH scavenging assay and HRBC membrane stabilization methods were performed for antioxidant and anti-inflammatory potential respectively.

Results: The pharmacological studies revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical. The IC\(_{50}\) values by DPPH scavenging assay observed for standard and leaves were 97.29 µg/ml and 486.78 µg/ml respectively. Thus, this plant extracts have significant antioxidant effect. It also had moderate anti-inflammatory activity. The IC\(_{50}\) values for anti-inflammatory activity by standard and coconut leaves were 21.46 µg/ml and 831.21 µg/ml respectively. These findings suggest that Cocos nucifera (L.) may be a possible source for the development of a new anti-inflammatory drug.

Conclusion: The phytochemical analysis of methanolic extract of coconut leaves showed that they contained significant presence of flavonoids, phenols, saponins, terpenoids and triterpenes. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols which was more than tannin content.

Keywords: Antioxidant, anti-inflammatory, Cocos nucifera, IC50 values, phenols, tannin content.
Higher plants contribute no less than 25% of the total drug market. Over the last 40 years, many potent drugs have been derived from flowering plants, including for example, D. pereirae, a species from which all anovulatory contraceptive agents have been derived; reserpine and other anti-hypertensive and tranquilizing alkaloids from the Rauwolfia species; pilocarpine to treat glaucoma and ‘dry mouth’, derived from a group of the genus Cuscuta. Although discovered through serendipitous laboratory observation, three of the major sources of anti-cancer drugs on the market or completing clinical trials are derived from North American plants used medicinally by native Americans: the papaw (Asimina spp.); the western yew tree (Taxus brevifolia), effective against ovarian cancer and may apple (Podophyllum peltatum) used to combat leukaemia, lymphoma lung and testicular cancer. Cocos nucifera (L.) is originally from Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. From that region, the fruit of the coconut palm is believed to have been brought to India and then to East Africa. After the discovery of the Cape of Good Hope, this plant was introduced into West Africa and, from there, dispersed to the American continent and to other tropical regions of the globe. C. nucifera has been called the ‘tree of life’ or ‘tree of heaven’ because of its value as provider of so many useful products. This species provides food, water, oil, medicine, fibre, timber, and fuel for many people living on islands in the Pacific Ocean.

Table 1: Total phenolic content (TPC) of Cocos nucifera leaves by using Folin and Ciocalteu reagent

| Absorbance | TPC (mg of GAE/g) | Average | TPC (mg of GAE/g)±SEM |
|------------|------------------|---------|-----------------------|
| 0.202      | 26.306           |         |                       |
| 0.209      | 27.435           | 26.951  | 26.951 ± 0.33         |
| 0.207      | 27.113           |         |                       |

South American trees (Pilocarpus spp.) in the Citrus family; two powerful anti-cancer agents from the Rosy Periwinkle (Catharanthus roseus); laxative agents from Cassia sp. and a cardiotonic agent to treat heart failure from Digitalis species.

Table 2: Total tannin content (TTC) of Cocos nucifera leaves by using Folin Ciocalteu reagent

| Absorbance | TTC (mg of TAE/g) | Average | TTC (mg of TAE/g)±SEM |
|------------|------------------|---------|-----------------------|
| 0.364      | 1.585            | 1.577   | 1.577 ± 0.010         |
| 0.358      | 1.557            |         |                       |
| 0.365      | 1.590            |         |                       |

Materials and Methods

In this study, all the chemicals, reagents used here provided from laboratory of Department of Pharmacy, USTC which source from Merck Limited, Mumbai, India and were analytical grade, pure and sorted under optimum storage conditions. Moreover, the drug mixtures and solutions were prepared accurately in standard volumetric flasks about one hour prior to obtain and recording the data.

Table 3: Test of different metabolites

| Secondary metabolites | Name of the test | Results |
|-----------------------|------------------|---------|
| Alkaloids             | Wagner test      | ++      |
| Flavonoids            | Specific test    | +++     |
| Glycosides            | General test     | ++      |
| Phensols              | Citrus test      | +++     |
| Saponins              | Proth test       | +++     |
| Tannins               | Ferric chloride test | ++       |
| Terpenoids            | General test     | +++     |
| Triterpenes           | Salkowski’s test | +++     |

Although discovered through serendipitous laboratory observation, three of the major sources of anti-cancer drugs on the market or completing clinical trials are derived from North American plants used medicinally by native Americans: the papaw (Asimina spp.); the western yew tree (Taxus brevifolia), effective against ovarian cancer and may apple (Podophyllum peltatum) used to combat leukaemia, lymphoma lung and testicular cancer. Cocos nucifera (L.) is originally from Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. From that region, the fruit of the coconut palm is believed to have been brought to India and then to East Africa. After the discovery of the Cape of Good Hope, this plant was introduced into West Africa and, from there, dispersed to the American continent and to other tropical regions of the globe. C. nucifera has been called the ‘tree of life’ or ‘tree of heaven’ because of its value as provider of so many useful products. This species provides food, water, oil, medicine, fibre, timber, and fuel for many people living on islands in the Pacific Ocean.

Figure 1: Graphical representation of anti-inflammatory activity of leaves of Cocos nucifera

Figure 2: Graphical representation of anti-inflammatory activity of standard.

Total Phenolic Content (TPC)

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu’s reagent is used in this ionized phenolic solution, the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu’s reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the color change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound.

Figure 3: Comparative study based on IC50.

The total phenolics of the extracts were determined using the Folin and Ciocalteu reagent, following the method used in previous study. The test sample (0.2 mL) was mixed with 0.6 mL of water and 0.2 mL of Folin-Ciocalteu’s phenol reagent (1:1). After 5 min,
1ml of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 3ml with distilled water. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue color from different samples was measured at 760 nm.14

**Figure 4: Antioxidant activity of leaves of Cocos nucifera by DPPH SCV assay.**

Total Tannin Content (TTC) determination
Fifty micro liters (µl) of tannins extract for each sample was taken in test tube and volume was made to 1.0 ml with distilled water. Then, 0.5ml Folio Ciocalteu reagent was added and mixed properly. Then 2.5ml 20 per cent sodium carbonate solution was added and mixed it and kept for 40 minutes at room temperature. Optical density was taken at 725nm in spectrophotometer and concentration was estimated15. Tannic acid was used as standard and tannin contents were measured as tannic acid equivalent.

**Figure 5: Antioxidant activity of standard by DPPH SCV assay**

**Anti-inflammatory activity**
Percent inhibition of protein denaturation was calculated as follows16:

\[
\text{% inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100
\]

The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect.

**Anti-oxidant activity**
The free radical-scavenging activity of extracts was evaluated with the DPPH assay based on the measurement of the reducing ability of antioxidants toward the DPPH radical17,18.

**RESULTS AND DISCUSSION**
The following tests were done to find the presence of the active chemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids and triterpenes is shown in Table 3. Due to the different chemical compositions present in a Cocos nucifera (L.) are obviously responsible for its different therapeutic and pharmacological activities.

In this study, the different constituents of the Cocos nucifera (L.) which are found should have some relationship with domestic medicinal applications.1 It should be mentioned here that the presence of these kinds of chemical constituents, it is expected that the selective plant Cocos nucifera should have anti-inflammatory activity and anti-oxidant activity. The method of HRBC membrane stabilization was chosen to evaluate anti-inflammatory effect. It is already proved that membrane stabilization of RBC is as effective as healing inflammation in provoking delayed hypersensitivity 9.

**Figure 6: Comparative antioxidant activity by DPPH SCV assay**

From Table 4, it is observed that the degree of membrane stabilization was increased by increase in concentration. That means the drug will give required action at higher concentration. As shown in Figure 2, it is observed that in Comparative % inhibition of protein denaturation, the Cocosnucifera leaves under the study of methanolic extract from 1.56±0.5% to 53.53±0.48% has shown moderate inhibition of protein denaturation at any concentration compared to the standard drug Diclofenac Sodium from 79.51±0.46% to 93.47±0.19%. It revealed that the plant extracts may have moderate anti-inflammatory effect which is probably mediated by HRBC membrane stabilization. The secondary metabolites such as phenolic compounds and tannins which were found in preliminary phytochemical screening might be responsible for such type of activity. The Antioxidant activity was performed by the method of free radical-scavenging assay. The antioxidant potential of the methanolic extract was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picyl hydrazyl (DPPH) free radical. So, the free radical-scavenging activity of extracts was evaluated with the DPPH assay.
based on the measurement of the reducing ability of antioxidants toward the DPPH radical\textsuperscript{17,18}. Here Table 7 shows that the absorbance of methanolic extract and Standard drug Ascorbic Acid decreased with the rise in their concentrations. As shown in Comparative % SCV of DPPH, methanolic leaves extract from 62.5 µg/ml to 2000 µg/ml exhibited % SCV ranging from 7.40±0.51% to 94.46±0.39% whereas Standard drug Ascorbic Acid from 62.5 µg/ml to 2000 µg/ml exhibited % SCV ranging from 61.90±0.30 % to 96.91±0.54 %. Thus the DPPH SCV capacity of the methanolic extract is close to the Standard drug Ascorbic Acid SCV capacity. This indicates good antioxidant power of the experimental extract which is shown in Figure 7. So, it revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation.

### Table 4: Spectroscopic Determination of anti-inflammatory activity of leaves of *Cocos nucifera*

| Concentration (µg/ml) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | IC50 (µg/ml) |
|-----------------------|------------|--------------|---------|--------------------|--------------|
| 125                   | 0.443      | 1.34         | 1.56    | 1.56±0.5           |              |
| 0.446                 | 0.467      | 2.67         |         |                    |              |
| 0.437                 | 0.405      | 9.80         | 11.43   | 11.43±0.9          | 831.21       |
| 0.391                 | 0.397      | 12.92        |         |                    |              |
| 250                   | 0.239      | 46.77        | 46.77   | 46.77±0.5          |              |
| 0.235                 | 0.243      | 47.66        |         |                    |              |
| 500                   | 0.213      | 52.56        | 53.53   | 53.53±0.48         |              |
| 0.207                 | 0.206      | 53.90        |         |                    |              |
| 1000                  | 0.123      | 54.12        |         |                    |              |

### Table 5: Spectroscopic Determination of anti-inflammatory activity of standard compound (Diclofenac-Na)

| Concentration (µg/ml) | Absorbance | % Inhibition | Average | % Inhibition ± SEM | IC50 (µg/ml) |
|-----------------------|------------|--------------|---------|--------------------|--------------|
| 125                   | 0.243      | 45.88        | 46.62   | 79.51 ± 0.46       |              |
| 0.239                 | 46.77      |              |         |                    |              |
| 0.237                 | 47.22      |              |         |                    |              |
| 250                   | 0.161      | 64.14        | 64.07   | 85.97 ± 0.25       | 21.46        |
| 0.159                 | 64.59      |              |         |                    |              |
| 0.164                 | 63.47      |              |         |                    |              |
| 500                   | 0.101      | 77.51        | 76.91   | 89.31 ± 0.46       |              |
| 0.107                 | 76.17      |              |         |                    |              |
| 0.103                 | 77.06      |              |         |                    |              |
| 1000                  | 0.057      | 87.31        | 87.45   | 93.47 ± 0.19       |              |
| 0.053                 | 88.20      |              |         |                    |              |
| 0.059                 | 86.86      |              |         |                    |              |

### Table 6: Comparative % inhibition of protein denaturation

| Concentration (µg/ml) | Leaves | Standard |
|-----------------------|--------|----------|
| 125 µg/ml             | 1.56   | 46.62    |
| 250 µg/ml             | 11.43  | 64.07    |
| 500 µg/ml             | 46.77  | 76.91    |
| 1000 µg/ml            | 53.53  | 87.45    |

### Table 7: Spectroscopic determination of antioxidant activity of leaves of *Cocos nucifera*

| Concentration (µg/ml) | Absorbance | % SCV | Average | % SCV±SEM | IC50 (µg/ml) |
|-----------------------|------------|-------|---------|----------|--------------|
| 62.5                  | 0.803      | 10.38 | 10.71   | 7.40±0.51|              |
| 0.806                 | 10.04      |       |         |          |              |
| 0.791                 | 11.72      |       |         |          |              |
| 125                   | 0.679      | 24.22 | 24.67   | 20.20±0.26|              |
| 0.675                 | 24.67      |       |         |          |              |
| 0.671                 | 25.11      |       |         |          |              |
| 250                   | 0.425      | 52.57 | 52.86   | 52.86±0.54| 486.78       |
| 0.429                 | 52.12      |       |         |          |              |
| 0.413                 | 53.91      |       |         |          |              |
| 500                   | 0.291      | 67.52 | 67.52   | 67.52±0.26|              |
| 0.287                 | 67.97      |       |         |          |              |
| 0.295                 | 67.08      |       |         |          |              |
| 1000                  | 0.107      | 88.06 | 88.91   | 88.91±0.46|              |
| 0.093                 | 89.62      |       |         |          |              |
| 0.098                 | 89.06      |       |         |          |              |
| 2000                  | 0.049      | 94.53 | 94.46   | 94.46±0.39|              |
| 0.056                 | 93.75      |       |         |          |              |
| 0.044                 | 95.09      |       |         |          |              |
CONCLUSION
From this research work it was found that qualitative evaluations show significant presence of flavonoids, phenols, saponins, terpenoids and triterpenes. Because each part of C. nucifera has different constituents, the pharmacological effects of the plant vary according to the part of the plant evaluated. Alkaloids, glycosides and tannins are also moderately present. Quantitative evaluations show significant presence of phenols than tannin content. The IC$_{50}$ values by DPPH scavenging assay observed for standard and leaves were 97.29µg/ml and 486.78µg/ml respectively. So, there is an excellent antioxidant activity in the methanolic extract. There is also moderate anti-inflammatory activity in the methanolic extract of coconut leaves. The IC$_{50}$ values for anti-inflammatory activity by standard and coconut leaves were 21.46µg/ml and 831.21µg/ml respectively.

CONFLICT OF INTEREST
No conflict of interest associated with this work.

AUTHOR’S CONTRIBUTION
The manuscript was carried out, written, and approved in collaboration with all authors.

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| Concentration (µg/ml) | Absorbance | % SCV | Average % SCV | % SCV±SEM | IC50 (µg/ml) |
|-----------------------|------------|-------|---------------|-----------|-------------|
| 62.5                  | 0.343      | 61.72 | 61.90         | 61.90±0.30|             |
| 125                   | 0.257      | 71.32 | 70.76         | 70.76±0.36|             |
| 250                   | 0.195      | 78.24 | 78.83         | 78.83±0.49| 97.29       |
| 500                   | 0.119      | 86.72 | 87.24         | 87.24±0.27|             |
| 1000                  | 0.047      | 94.75 | 94.49         | 94.49±0.16|             |
| 2000                  | 0.021      | 97.66 | 96.91         | 96.91±0.54|             |