CHAPTER 2

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Regular items are a wellspring of new concoction differences. The wellsprings of normal item are plants, creatures and microbes. Amongst them, plant and its derivatives are further solid for its sustainability and considered as impetus for human welfare. Still, these sources are principally required materials for human services framework in a few sections of the globe. Subsequently, in the previous couple of years, there is a developing examination enthusiasm for plants as a remedial operator. The remedial capability of plant items can be followed back to more than 5000 years prior as there is confirmation of its utilization in the treatment of maladies and for rejuvenating body frameworks in Indian, Chinese, Egyptian, Roman and Greek civic establishments. India is one among the super differences problem areas with rich legacy of customary learning of people prescriptions. In this manner, plants having restorative capacity are generally utilized by most segments of individuals both as people drugs in various indigenous frameworks of medication like Ayurveda, Siddha and Unani and furthermore as prepared result of pharmaceutical industry. India has around 4.5 million plant species and among them just 2,50,000 - 5,00,000 plant species have been researched phytochemically for organic or pharmacological movement. The potential for forming phytomedicine into different social insurance items seems fulfilling, both from the point of view of economy and wellbeing. Friedman et al. (2007) [21] and Serafino et al. (2008) [22] noticed that many plant concentrates are very successful than the manufactured ones with no or irrelevant symptoms and next to no logical research on their natural
action has been worked out. Besides, the development of multidrug safe microbial strains which are restricting the adequacy of engineered medications prompted mindfulness among the average folks against the utilization of manufactured item. This element constrained the researchers to create techniques for bioprocesses for the generation and extraction of mixes from regular inexhaustible hotspots for their potential use in sustenance, beauty agents and medicine industry with antimicrobial, cell reinforcement and mitigation. Consequently, phytochemicals usage as major bioactive compound with more advantages is picking up energy.

2.1.1 Experimental Medicinal plants

2.1.1.1 Acalypha alnifolia

*Acalypha alnifolia* belongs to *Acalypha* genus which contains approximately 513 species and belongs to the Euphorbiaceae, family. This plant is predominant in few parts of Kerala like Kozhikode and Palakkad; and in Tamilnadu, it was present in Tiruchchirappalli, Viluppuram, Dindigul, Namakkal, Tiruvannamalai, Salem, Kancheepuram and Tirunelveli. The leaves and the juices are used as a medicine to treat dysentery. The whole plant is useful in curing asthma, pneumonia and bronchitis. The leaves are used in treating maggot-eaten sores. The plant roots were utilized as a cathartic [23]. Figure 2.1 shows the image of *Acalypha alnifolia*.

![Acalypha alnifolia](image.jpg)

*Figure 2.1 Acalypha alnifolia*
2.1.1.2 **Dregea volubulis**

*Dregea volubulis* is a plant species that belongs to the Apocynaceae family and is native to India. Sneeze Wort is a common name of *Dregea volubilis*. Dregea is smooth, short and possess woody vine. Flowers are arranged interpetiolar or axillary. They are green or yellowish green in colour with nice fragrance. The leaves of this plant are roughly round or ovate in shape, of 5-10 cm breadth and 7.5-15 cm length, round and pointed at base and tip. The plant seeds are sharp-edged, concave, elliptic, and smooth and capped with white, fine, silky hairs. The follicles appear as turgid, ribbed, lengthy and velvet till maturation. The flowering of this plant usually takes place in April-September. The tender stalks and roots are considered cough medicine and emetic. The leaves of this plant are much used to treat abscesses and boils. The early roots are dissected and the juice expelled outside is introduced into the nose to cause sneezing [24].

![Figure 2.2 Dregea volubulis](image)

2.1.1.3 **Ipomoea staphylina**

*Ipomoea* is the largest genus from Convolvulaceae family consisting over 500 species. These plants are seen in India, Sri Lanka and China. December-March is the flowering period of this plant. Its common name is morning glory. Humans
utilize Ipomoea for their medical and psychoactive compounds, mainly alkaloids. Certain species are well-known for their properties in folk medicine; for instance Tampico jalap (I. simulans) is employed to produce jalap, a therapeutic preparation accelerating the passage of stool.

![Ipomoea staphyliana](image)

**Figure 2.3 Ipomoea staphyliana**

### 2.1.1.4 Blepharis maderaspatensis

Currently, the discovery of new plant based drugs which can be used in the treatment of several diseases has been of keen interest to researchers. Thus, in this study, different extracts of two medicinally important species have been selected to evaluate their antioxidant activity. *Blepharis* is an Afro-asiatic genus belongs to the family Acanthaceae. It comprises 129 species which occur in arid and semiarid habitats [25]. *Blepharis maderaspatensis* (L.) Heyne ex Roth. (Acanthaceae) is frequently utilized by hyperlipidaemic patients in India as an alternate therapeutic tool to treat hyperlipidaemia. It is also considered as a traditional medication as a wound healing agent and diuretic. The plant extract is employed to treat ailments like bone fracture, diarrhea, boils and lactation [26]. *B. maderaspatensis* is used for headache.
Seeds of this plant are used to treat nervous system diseases and also act as an aphrodisiac and diuretic [27], it is used to cure wounds and cuts [28], and juice extracted from leaf is warmed with ginger oil and applied on affected places to rectify wound [29]. Plant seeds comprise steroids and it is also used to treat brain disorders [11]. *Blepharis molluginifolia* leaves are creased and the leaf extract is spread on head to treat head ache [12]. It is utilized to treat skin diseases, bone fractures, allergies and urinary discharges [13]. In case of joint pains, flat branches of the plant is warmed and tied. Leaves are roasted and then extract is obtained, this product is drunk as a medicine against flatulence. Roots are employed as antidote on snake- bite [14]. On revising the reviews and the studies of the herbs *Blepharis maderaspatensis* and *Blepharis molluginifolia* till date, it has very less information regarding the bioactivities, even though they are potent medicinal herbs. Thus in the present study, an effort has been made to analyze the antioxidant capacity of the above mentioned herb.

![Blepharis maderaspatensis](image)

Figure 2.4 *Blepharis maderaspatensis*

### 2.2 PHYTOCONSTITUENTS

Phytoconstituents are a variety of compounds produced by plants. Researchers have estimated that there are around 4000 phytochemicals among which only 25% have been studied so far. The phytochemicals in common use are
flavanoids, terpenoids, tannin, saponin, carotenoids, anthocyanidins, polyphenols, quinones, catechins etc [30]. Sugars, organic acids, minerals, dietary fiber, and bioactive constituents like alkaloids, flavanoids and phenols are derived from plant metabolites. These compounds are produced as plant metabolites prevailing in plants in twenty six forms, whereas other compounds exist as precursors in inactive form. During pathogen attack or tissue damage these precursors gets activated and these plant based metabolites comes with a variety of physical, chemical and biological activities. Most of the compounds are appropriate to a number of families, and they have their own specific organizational features.

In ancient times, the medicines and drugs were obtained from plant sources, in two ways: a) from parts of the plant in simple form and b) from crude plant extract in complex form. Nowadays, a significant amount of medicines are derived from plants that work very well against several diseases. These plant based compounds are isolated in crude form the medicinal plant and modified according to the efficacy of the drug. In the developed countries, 25% of the medicines are plant-based and the usage of therapeutic plants is common among the native people in countryside parts of many developing nations. In older days, our descendants tried out new drugs by trial and error method using medicinal plants. Though few curative properties credited to plants have confirmed by the investigators as invalid ones. In developing countries, due to the worse sanitation facilities wound infection is predominantly observed. Wounds are basically injuries that takes place when there is a cut or break in the skin. The suitable wound healing method is important in re-establishment of interrupted anatomical continuity and disturbed functional status of the skin.

Wound healing is initiated from the time of injury and might carry on for different periods of time subject to the level of wounding and the procedure can be generally classified into 3 stages phases namely a) inflammatory phase b) proliferate phase and c) remodeling phase which finally decides the power and look of the healed tissue. Wound healing procedure generally involves inflammation,
coagulation, granulation tissue formation, connective tissue remodeling, matrix formation, aquisation of wound strength and collagenization. Research on wound healing mediators is an interesting and emerging area in modern pharmaceutical sciences and several conventional specialists around the globe predominantly in nations like China and India have appreciable data of several least-known hitherto unknown wild plants for curing burns and wounds. In Africa and Asia, conventional ways of treatment and plant based medicines are tested scientifically for wound associated diseases.

2.2.1 Flavonoids

Flavonoids are a class of plant derived compounds originated in therapeutic plants that are reported to apply effective antioxidant activity against the superoxide radical [31]. They provide the pigments in the fruits, flowers and seeds. Flavonoids are generally classified into six namely flavones, chalcones, flavandiols, flavonols, anthocyanins and proanthocyanidins [32]. They are strong water-soluble free radical scavengers that have robust anticancer activity, inhibit oxidative cell damage and defend against all types of cancer. Flavonoids are well-known to decrease the heart diseases risk [33]. The major functions of flavonoids are associated to their ability to intermingle with enzymes via protein complex and cytotoxicity. Certain flavonoids offer stress protection, for example, acting as free radicals scavengers such as reactive oxygen species (ROS), and also as chelating metals that produce ROS by means of the Fenton reaction [34].

2.2.2 Tannins

Tannins are a group of phenolic substances which has the ability to tan leather. They are existent in most parts of the plant namely wood, bark, fruits, roots and leaves. Tannins possess numerous human physiological activities, such as phagocytic cells stimulation, anti-infective actions and host-mediated tumor activity [35]. These metabolites are found in a range of plants used as food and feed. These
possess food grains such as millets, sorghum, dry beans, barley, winged beans, pigeonpeas, peas, carobs, and other legumes[36, 37, 38]. The antioxidative property of tannins may be correlated to their anticarcinogenic and antimutagenic activity in defending cellular constituents from oxidative damages which includes lipid peroxidation, DNA single-strand breakage and 8-hydroxydeoxyguanosine formation. For instance, quercetin had been confirmed to prevent the superoxide anions generation by neutrophils [39].

2.2.3 Polyphenols

Polyphenols are derived from benzene with the chemical formula C₆H₅OH and are considered to be toxic to the pathogens. Polyphenols play a vital role in inhibiting the mutagens expression and inactivating cancer-causing agents [30, 33]. They occur predominantly in fruits, cereals and vegetables. Polyphenols are used as anticancer mediators because of the following properties: high accessibility, specificity of the response, less toxicity, and different biological functions. One more advantage of polyphenols is that they tend to act as a cytoprotective agent to the normal cells and show cytotoxic effect against malignant cells [40]. Polyphenols usually initiate apoptosis via pro-oxidative action that is applied rather than their antioxidative action relying upon their concentration and environmental conditions [41]. Therefore, they may interface contrastingly contingent upon the cell sort: healthy vs cancer-causing one. Furthermore, polyphenols aid to set up the body's immune system by repressing angiogenesis important for tumor development. In any case, the polyphenols bioavailability symbolizes a major obstacle as they just achieve the objective organs in low concentrations [42].

2.2.4 Saponins

Saponins are a distinctive group of glycosides that possess soapy characteristics and also act as active antifungal agents [43]. Saponins are also found in Sapindaceae family, with its Sapindus genus, and in the closely related families
Aceraceae (maples). Saponins are endorsed commercially as dietary supplements and food ingredients. Licorice extract and its chief saponin, glycyrrhizin are used as a medicine, sweetener and flavor enhancer in foods [44]. Some interesting biological applications of saponins include a) anti-inflammatory agent [45], b) hypocholesterolemic agent [46] and c) inducing immunity [47].

2.2.5 Carotenoids

Carotenoids are tetraterpenoids that are synthesized in plants, algae and bacteria. Carotenoids stimulate phase I & phase II metabolic enzymes that possess a vital role in the carcinogen detoxification [48]. They are found in coloured vegetables and fruits. Carotenoids play a role as antioxidants and stimulate oxidative stress resistance [49, 50]. Many carotenoids are used in cancer prevention. Recent reports on anticancer activity reveal consumption of carotenoid rich vegetables and fruits reduce the risk of cancer and also develop the immunity [51]. It is reported that β-carotene aids in neutralizing the free radicals that are responsible to cause cancer. Certain studies with laboratory animals have reported that β-carotene can delay or avoid initiation of skin cancer and sarcomas in carcinogens exposed mice [52].

2.3 ANTIOXIDANTS

In food industry, the antioxidants are characterized as substances in less size that are capable of preventing or retarding the fats oxidation [53]. During catabolism in living organisms, oxidation is necessary to obtain energy. Nonetheless, cell death and tissue damage may be the outcomes due to the presence of oxygen-centred free radicals and other reactive oxygen species in the body. These free radicals play a vital role in the study of certain diseases like Alzheimer’s disease, cardiovascular disease, Parkinson’s syndrome and Cancer [54, 55]. Antioxidants are usually constant molecules that possess the ability to provide an electron to a free radical and lessen its capacity of cell damage by neutralizing it.
The cell damage is majorly done by antioxidants via the radical scavenging property. The molecular weight of antioxidants is low and they can securely cooperate with free radicals and end the chain response before fundamental particles are harmed. Glutathione, uric acid and ubiquinol are the examples of antioxidants produced during normal metabolism in the body. Free radical scavenging in the body is done by several enzyme systems. They are the main micronutrients like vitamins for instance vitamin C (ascorbic acid), vitamin E (α-tocopherol) and β-carotene. These micronutrients cannot be manufactured in our body; and thus they should be supplied via the diet [56]. The greater production expenses and poor efficacy of regular cancer prevention agents, for example, tocopherols, together with the expanding awareness of users as to nourishment added substance security, made a requirement for distinguishing alternate natural and most likely more secure sources of food antioxidants [57]. The most normally utilized cancer prevention agents currently are butylatedhydroxy toluene (BHT), propyl gallate (PG), butylated hydroxyanisole (BHA) and tert-butylhydroquinone (TBHQ). Be that as it may they are associated with being in charge of liver harm and carcinogenesis in research animals. Hence the improvement and usage of more secure natural cancer prevention agents are required [58]. Examinations on antioxidants sources have turned into an essential research range in most recent couple of years. These new antioxidant sources could be utilized for direct utilization or for the generation of nourishment supplements that will help to enrich the nutritional value of foods [59].

2.3.1 Inflammation

Inflammation is portion of the intricate innate immune reaction of tissues to harmful stimuli, for example, harmed cells, pathogens, or irritants and it may be categorized into acute and chronic inflammation. Inflammation is a protective reaction that includes blood vessels, immune cells and molecular bodies. The main roles of inflammation are listed out in the following points:
a) Removal of necrotic cells and tissues injured from the inflammatory process, rudeness

b) Elimination of initial reason of cell harm and

c) Initiation of tissue restoration

Inflammed macrophages discharge a wide scope of mediators for example, some pro-inflammatory cytokines like IL-1, TNF-α, IL-12, IL-8, IL-6, reactive oxygen species, prostaglandins and nitric oxide and they are associated in the inflammatory response, section of the innate immune defense. Chronic inflammatory diseases such as rheumatoid arthritis occur when the host tissues are damaged due to the release of these biomolecules during the course of acute or chronic infection [53]. Inflammation is usually induced by foreign particles such as viruses, bacteria and parasites. Along these lines the fiery reaction is vital responses that secure us and it is described by inflammation, warm, swelling, torment and capacity loss. The reaction given by the body in terms of inflammation indicates the effective removal of contamination and curative of the harmed cells. Though, in certain circumstances the wound is not removed and as a result prolonged inflammation progresses which is responsible for the extreme tissue damage and might prompt to advancement of granulomas [54]. It is critical for the organism to identify if there are additional microbes in a lesion and to avoid the dispersion of these, regardless of the possibility that the outcome is extra tissue injury. Consequently tissue damage on its own starts 2 kinds of triggering signals in the immune system; (i) neurons react to ache through arrival of active protein subunits [55] and (ii) constituents for example, HMGB1 and mitochondrial peptides are discharged from the fragmented cells [56].

Histamine, chemokines, proteases and cytokines are the various substances delivered by Macrophages and Mast cells during the course of the signaling pathway. Amongst different molecules vasodilatation and extravasation of fluid is performed by histamine. This creates a feasible way for cells and proteins to
pass through the tissue. The neutrophiles consume microbes through phagocytosis or discharges toxic chemicals and cytokines when they are travelling from blood to the inflamed tissue. The gathering of cells and fluid makes the distinctive indicators of inflammation; redness, heat, swelling and ache. The neutrophils and macrophages are the major inflammatory forms and lymphocytes take an interest just later in the inflammatory reaction [57].

Modern healing methodologies for curing bone illnesses concentrate on inhibiting the TNF-α cytokine by administrating the neutralized antibodies or antagonists [58]. Notwithstanding, it is intriguing to estimate that cytokines engaged in the determination of inflammation may be appropriate marks for curing long-lasting inflammation later on. Ongoing inflammatory reactions are regularly related with allergies, some bacterial contamination, autoimmune diseases and injuries are difficult to treatment and several methods are carefully examined. Instances of curative mediators utilized as a part of inflammatory systems are; (i) mediators that lessen leukocyte extravasation such as nullifying antibodies to the leukocyte functional antigen (LFA)-1 [59] (ii) non-steroidal anti-inflammatory drugs (NSAIDs). and (iii) corticosteroids.

Amongst these, the powerful anti-inflammatory agents are corticosteroids with different effects comprising the restraint of chemotaxis, cytotoxicity, phagocytosis, and down regulation of cytokine gene expression [25]. Besides, reports have shown that IL-2 gene transcription can be prevented by corticosteroids when they are meddling with certain elements like NF-AT and AP-1 [26]. Furthermore, the NF-κB inhibitor IκBa protein transcription was incited by steroid hormones resulting in NF-κB action hinderance [27]. As a final point corticosteroids prevent adhesion and movement of inflammatory cells through down regulation of fragments like LFA-1, E-selectin, and ICAM-1 [28].

On the whole corticosteroids possess a noteworthy role in therapeutic medications of inflammatory conditions today. In any case, several clinically
utilized anti-inflammatory agents aim and prevent pro-inflammatory cytokine roles [29]. They inhabit many side effects on human health for example weakened bones and unusual fat buildup to the composites. This is a prerequisite for numerous conditions that they are recommended in contradiction. In this manner, potential novel medications with a smaller amount damaging side-effects are required for curing inflammatory diseases.

**Mediators of Inflammation:**

a) Vasoactive amines (Histamine, Serotonin)

b) Platelet activating factor (PAF)

c) Complement system

d) Kinin system

e) Cytokines

f) Nitric oxide

g) Adhesion Molecules

h) Arachidonic acid metabolites:

• Prostaglandins (PGs)

• Thromboxane A2 (TXA2)

• HETE (hydroxy-eicosatetraenoic acid)

• Leukotrienes (LTs) mediated by cyclooxygenases (COX)
2.3.2 Apoptosis

The biochemical hallmarks of apoptosis are release of specific mediators into cytoplasm by mitochondria, activation of caspase cascade and alterations in membrane symmetry. The cell is consequently fragmented into minor membrane-enclosed portions (apoptotic bodies), that are removed via macrophages [10]. Defects in apoptosis play a crucial role in tumor development as well as chemo resistance of cancer cells.

There are two principle pathways that prompt apoptosis, a) intrinsic pathway and b) extrinsic pathway. The intrinsic apoptotic pathway (mitochondrial) begins when an injury occurs within the cell and the extrinsic pathway also called as death receptor pathway is stimulated by death receptors (DRs) ligation communicated at the cell surface [60].
2.3.3 Anticancer – activity

The free radicals accumulation in tissues or organs might lead to oxidative damage to biomolecules and cell membrane, in the long run prompting several chronic diseases, for example, cancer, diabetes, cardiac dysfunction, inflammatory disorders and degenerative disorders [62]. Phagocyte cells liberate free radicals and these are significant in inflammatory progressions, since they are related in the nuclear factor kB activation, which prompts the cyclooxygenase 2 and inflammatory cytokines transcription [63].

Matrix metalloproteinases (MMPs) basically include membrane-bound endopeptidases and secreted endopeptidases which disintegrate extracellular matrix proteins. Based on the substrates favoured by the enzymes, MMPs can be categorized into stromelysins, collagenases, membrane-type matrix metalloproteinases and gelatinases [64]. Collagenase 3 (MMP-13) was portrayed to be complicated in the enhancement and metastasis of lung cancer and breast cancer.
Likewise, collagenase assumes a vital part in osteoarthritis and rheumatoid arthritis [65].

Plant derived compounds and their semi-synthetic derivatives keep on playing a vital part in anticancer medication treatment [66]. These consist of vinblastine, topotecan and irinotecan, vincristine, etoposide, resulted from epipodophyllotoxin and paclitaxel (taxol).

2.3.4 Anticancer and Anti-inflammatory drugs – A brief note

Anticancer drugs

Though Surgery and Radiation therapy seems to be a part of cancer treatment, Chemotherapy has been widely used to treat cancer. The main merit of chemotherapy is they can target cancer cells throughout the body unlike in contrast to radiation and surgical methods that are localized treatments [67]. In anticancer drug therapy, the plant secondary metabolites and their partially derived byproducts endure to play a vital role [66]. This consists of vincristine, vinblastine, topotecan and irinotecan, paclitaxel (taxol) and etoposide, derivative of epipodophyllotoxin. Anticancer drugs can be classified into three types namely, a) Targeted drugs, b) Cytotoxic drugs and c) Hormonal drugs. Table 2.1 shows the types of drugs.

Table 2.1 Types of Drugs

<table>
<thead>
<tr>
<th>Targeted drugs</th>
<th>Cytotoxic Drugs</th>
<th>Hormonal Drugs</th>
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<tbody>
<tr>
<td>Angiogenesis inhibitors - Bevacizumab</td>
<td>Topoisomerase-2 inhibitor - Etoposide</td>
<td>Aromatase inhibitors – Anastrozole, Letrozole</td>
</tr>
<tr>
<td>Tyrosine protein kinase inhibitors - Nilotinib, Imatinib</td>
<td>Platinum coordination - Carboplatin, Cisplatin, Oxaliplatin</td>
<td>Glucocorticoids - Prednisolone</td>
</tr>
<tr>
<td>EGF receptor inhibitor – Erlotinib, Gefitinib</td>
<td>Topoisomerase-1 inhibitor – Irinotecan, Topotecan</td>
<td>GnRH analogues – Triotorelin, Nafarelin</td>
</tr>
<tr>
<td>Unarmed monoclonal antibody - Trastuzumab, Rituximab</td>
<td>Antibiotics: Actinomycin D, Doxorubicin, Daunorubicin, Epirubicin, Bleomycins, Mitomycin</td>
<td>Estrogens – Ethinylestradiol, Fosfestrol</td>
</tr>
</tbody>
</table>
Targeted drugs | Cytotoxic Drugs | Hormonal Drugs
---|---|---
Proteasome inhibitor - Bortezomib | Microtubule damaging agents – Docetaxel, Vinblastine, Paclitaxel, Vincristine, Vinorelbine, | Progestins - Hydroxyprogesterone acetate |
| Alkylating agents | Selective estrogen receptor modulators - Tamoxifen |
| Antimetabolites | 5-α reductase inhibitor - Finasteride |
| Miscellaneous: L-Asparaginase, Hydroxyurea, Arsenic trioxide, Tretinoin. | Selective estrogen receptor down-regulators Antiandrogen - Flutamide |

**Anti-inflammatory drugs**

The examples of anti-inflammatory drugs are classified into three:

a) Steroidal: ex. Corticosteroids (inh. gene transcription)

b) Non-steroidal Anti-inflammatory Drugs (NSAID) (inh. COX) Examples: ASA, indomethacin, ibuprofen

c) Selective COX-2 inhibitors

**NSAIDs**

a) NSAIDs inhibit synthesis of PGs which are the main factors playing a role in the inflammation.

b) Inhibit synthesis of PGs through inhibition of cyclooxygenase

c) Enzymes which are responsible for production of PGs

**2.4 PROTOCATECHUIC ACID**

Protocatechuic acid (3,4-dihydroxybenzoic acid) is a phenolic compound commonly distributed in nature. Like other simple phenolic acids, protocatechuic
acid is observed in most of the plant species, [69] such as the bran and grain brown rice [70] and onion [71]. It is noticed in many fruits, such as plums [72]; gooseberries [71]; grapes [73]; and nuts, such as almonds [74]. Studies using in vitro cellular system and ROS generation have shown that protocatechuic acid inhibits both the formation of free radicals, including the highly reactive hydroxyl radical, and the scavenging of free radicals [75]. The neutralization of free radicals is the result of their reaction with hydroxyl groups of protocatechuic acid. In vitro models showed that protocatechuic acid prevents oxidative DNA damage and lipid peroxidation [76]. Daily consumption of protocatechuic acid was not clearly defined, however the content in food rarely reaches 2-10 mg/g. Figure 2.7 shows the structure of protocatechuic acid and Figure 2.8 shows the Effect of protocatechuic acid on cytokinin expression levels of iNOS, COX2, TNF-α, IL1 and IL-6 in the macrophage RAW 264.7 cell line.

![Figure 2.7 Chemical structure of protocatechuic acid (Molecular weight: 154.12)](image)
Figure 2.8  Effect of protocatachuic acid on cytokinin expression levels of iNOS, COX2, TNF-α, IL1 and IL-6 in the macrophage RAW 264.7 cell line