CHAPTER- 7.
RESULT AND DISCUSSION

The objective of the present study covered the standardization, preliminary phytochemical investigation and immunomodulatory activity of the Bombax ceiba bark extract, and Averrhoa carambola leaves extract. As a part of standardization study, the macroscopical examination of bark and leaf was considered. Macroskopical evaluation is a technique of qualitative assessment based on the study of morphological and sensory profiles of drugs [1]. The result of the morphology of B.ceiba bark showed that pieces of bark were generally curved fragments, and the color was internally brown while externally it was gray in color. Bark fracture was fibrous odor was characteristics and taste was mucilaginous. The result of the morphology of A.carambola leaf showed that the leaf was compound, pinnate and alternate. The shape of the leaflet was ovate to ovate-lanceolate in shape, the margin was entire, the apex was acute base was oblique, the surface was glabrous, and the taste was bitter. The macroskopical characters of the plant can serve as diagnostic parameters.

The anatomy of bark and leaf were studied by taking transverse sections. The result of microscopical examination of bark showed the presence of cork, cork cambium, phelloderm region, medullary rays and stone cells. The T.S of the leaf through midrib in fig. 4.10 showed the presence of single layered epidermis covered with cuticle. The epidermal cell was oval in shape. Pointed trichomes were present and beneath the epidermal cells
cholenchymatous cells were present. Center region is covered by vascular tissue that was arch shaped. The microscopical studies were the distinguishing features and could be used as the anatomical marker.

The study of various parameters like ash value, extractive value, fluorescent analysis and loss on drying of the bark and leaf was done. The results exhibited greater extractive values in hot extraction, designating the effect of raised temperature on extraction. Calculation of percentages of the extractive values was done with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extract. The extractive values are also helpful in the estimation of specific constituents soluble in the particular solvent [2].

With the help of three different methods, the ash values were determined. Which measured total ash (bark 5.5, leaf 8.5), acid-insoluble ash (bark -1.68, leaf- 1.71), and water-soluble ash (bark- 2.26 and leaf- 4.13). The total ash is particularly important in the evaluation of purity of drugs. These ash values are important quantitative standards. Hence, ash determination furnishes a source for judging identity and cleanliness of a drug and gives information related to its adulteration with organic matter [2].

Observation of foreign matter, loss on drying, determination of pH of drug and powdered drug reaction was done, and the results are tabulated. In various solvents, the fluorescence analysis of the powdered drug from the bark of *B. ceiba* and leaves of *A. carambola* was done under normal and UV light. All the extracts were examined in short UV (254nm) and long UV (366 nm) to detect the fluorescent compounds. The observations are given in Table 4.19 and 4.41. Fluorescence is the phenomenon exhibited by various
chemical constituents present in the plant material. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives or decomposition products by applying different reagents [1].

The result of thin layer chromatography showed three spots in methanol extract and three spots in the aqueous extract of *B. ceiba* bark. While in *A. carambola* leaves extract thin layer chromatography result showed three spots in aqueous and four spots in methanol extract with different *R*<sub>f</sub> values.

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents. The chromatographic profile may deliver as a characteristic fingerprint for qualitative evaluation of bark and leaf.

The preliminary phytochemical screening of the plant material was done. Which involve successive solvent extraction with the help of different solvents. All the solvents were used in order of increasing polarity to obtain diverse polar and nonpolar phytoconstituents possessing different solubility pattern. Then different chemical tests were done for qualitative detection of the various class of chemical constituents. The results are given in Table 4.22 and 4.44. The result of the preliminary phytochemical investigation of *B. ceiba* showed that alkaloid was absent, and carbohydrate, glycoside, phenolic compound and tannins, flavonoids were present. Sterols were present while saponins and acidic compounds were missing in the extract. While the extract of *A. carambola* showed the presences of phytosterols, flavonoids, the acidic compound, carbohydrates, glycosides, and alkaloids.

The result of qualitative HPTLC fingerprinting of bark showed eight peaks and leaves showed twelve peaks with different intensities. Before starting
any further pharmacological and toxicological examination standardization of crude drugs are important. It is done just because of in the case of herbal drugs there is a considerable scope for variation of active ingredients. Variation may occur due to alteration of the place of cultivation, time of collection and method of storage [1]. Therefore, crude drugs are assessed by these methods, and these are the relevant parameters for quality determination. These findings will also be helpful to differentiate these species from the closely related other species. In spite of so many modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means [1].

The estimation of immunomodulatory activity of *B. ceiba* bark and *A. carambola* leaves was done by administration of test extracts by oral feeding cannula to the test groups by the model of intensive chemotherapy with cyclophosphamide. Hemagglutinating antibody (HA) titer, delayed type of hypersensitivity (DTH) response, hematological profile (Hb, WBC, RBC), proinflammatory cytokine estimation as well as relative organ weight were determined as a measure of immunomodulatory parameters. While different stress-related enzymes lipid peroxidations (LPO), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) were estimated as a part of the antioxidant activity.

From the result, it is evident that in haemagglutination test both the plant extracts (MEBC and MEAC) showed an increased response in all doses. Indicating protective effects on humoral immunity by significantly (P<0.001) increasing haemagglutination antibody titer value in a dose-
dependent fashion. MEBC and MEAC treatment to mice, significantly (P<0.001) protected them from CP-induced immunosuppression of humoral immunity. This protection showed the stimulatory effect of extracts over B-cells. This activity could be due to the presence of flavonoids that augment the humoral response by stimulating the macrophages and b-lymphocytes subsets employed in the antibody synthesis [3].

With the help of foot pad reaction method, delayed-type hypersensitivitiy was assayed. The result acknowledged that MEBC at a dose of 150 mg/kg and 300 mg/kg showed significantly (P<0.001) increased in DTH reactivity in mice. The MEAC at a dose of 800 mg/kg and 1200 mg/kg showed significantly (P<0.001) increased in DTH reactivity in mice when compared to control animals. The increased in DTH reaction in mice in response to cell dependent antigen revealed the stimulatory effect of B. ceiba bark extract and A. carambola leave extract on T cells. The DTH response, which directly correlates with cell-mediated immunity increases, the mechanism behinds this elevated DTH could be due to the sensitized T-lymphocytes. When they challenged by the antigen, they are converted to lymphoblast and excrete a variety of molecules along with proinflammatory lymphokines, attracting more scavenger cells to the site of reaction [4]. An increased in DTH response indicates the stimulatory effect of the plants which has occurred on the lymphocytes and accessory cell types needed for the expression of this reaction. The histology of DTH reaction can be different for different species, but the general characteristics are an influx of immune cells at the site of injection [5].
Effect of MEBC and MEAC on relative organ weight showed that no significant relative weight difference in the liver, kidney and the spleen were recorded in various groups of animals when compared to control group. But CP treatment caused a significant reduction in relative organ weight of spleen as compared to control group animals.

The majority of all the cells type involved in the immune system are produced from conventional hematopoietic stem cells of bone marrow. Bone marrow also supplies microenvironment for antigen dependent differentiation of B-cells. Since MEBC and MEAC increased circulating antibody titer, it was thought worthwhile to evaluate their effect on peripheral blood counts. Effect of plant extract on hematological parameters showed that MEBC significantly (p<0.05) increased the white blood cell count at the dose level 150 mg/kg and 300 mg/kg as compared to control group animals. At the same time MEAC treatment also showed significantly increased in white blood cell count at the dose level 800 mg/kg (p<0.05) and 1200 mg/kg (p<0.01) as compared to control group. But CP injection caused a significant (p<0.001) reduction in white blood cell count as compared to healthy control group animal. Although combined treatment of CP with MEAC and CP with MEBC showed significant restoration of bone marrow activity as compared to cyclophosphamide treated group. Hence both the tested extracts significantly increased WBC, counts. Both innate and adaptive immunity depends upon the actions of WBC. The innate and adaptive immune system together provides a remarkably effective defense system [7].
Cyclophosphamide is one of the best famous alkylating anticancer drugs that produced toxic side effects. Toxicities of cyclophosphamide include the suppression of bone marrow, vomiting, liver, renal, and bladder injury. Several visual symptoms, such as lethargy, alopecia, unusual weakness, and anorexia. During chemotherapy, cyclophosphamide also causes hepatic damage as well as neurotoxicity, which is a problem associated with internal organs due to its cytotoxic nature [8, 9]. In the evaluation of the immunomodulatory activity, maximum studies used agent’s similar to cyclophosphamide, cisplatin or corticosteroids to urge the immunosuppression in the experimental animals [10]. These agents are known to generate free radicals in the biological system and thereby cause oxidative stress [11]. The present study had shown that the administration of cyclophosphamide not only impair the immune responses but also produce oxidative stress in mice. It appeared that cyclophosphamide that is an active generator of superoxide radicals might reduce the immune response through oxidative stress. It further observed that administration of plant extract prevented the cyclophosphamide-induced changes of immunological and oxidative stress parameters. Hence, the immunomodulatory effect of plants may be subsequent to the antioxidant activity that it possesses.

Plant extract effects on proinflammatory cytokines level showed that the secretion of TNF-α and IL-6 significantly decreased (P<0.001) in the negative control group when compared to normal control group animals. While TNF-α and IL-6 level upregulated significantly (P<0.001) in MEBC different dose treatment as compared to normal control animals. In case of MEBC different dose along with CP treatment, a significant recovery of
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An immunosuppressive effect was found when compared to negative control animals. Even in case of MEAC different dose treatment increased was found in TNF-α and IL-6 level but it was not statistically significant when correlated to control group animals. Thus, MEBC showed the stimulatory effect of TNF-α and IL-6 cytokines while in MEAC treatment increased was found in TNF-α and IL-6 level but it was not statistically significant when compared to control group animals. The proinflammatory cytokines deal the inflammatory response in the body such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). The level and persistence of TNF-α and IL-6 cytokines play a significant role in determining the behavior of a given factor in immunomodulation. IL-6 plays a critical role in host immune responses, such as acute protein synthesis, and the maintenance of homeostasis also acts as both a pro-inflammatory and anti-inflammatory cytokine [12].

From these result, it has been cleared that the stimulatory effect produced by MEBA and MEAC extracts in humoral immune function and cellular immune function may be due to the cell-mediated and humoral antibody-mediated activation of T and B cells. This may account for its efficient use in the treatment of immune related disorders.
6.1. References


