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Microbiological Cytotoxic, Haematological and Histopathological Investigations of Seven Bitter Medicinal Plants of Bangladesh

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MICROBIOLOGICAL CYTOTOXIC, HAEMATOLOGICAL AND HISTOPATHOLOGICAL INVESTIGATIONS OF SEVEN BITTER MEDICINAL PLANTS OF BANGLADESH.

M. Phil. Thesis

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Submitted by

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DECLARATION CERTIFICATE

I certify that the thesis entitled "MICROBIOLOGICAL CYTOTOXIC, HAEMATOLOGICAL AND HISTOPATHOLOGICAL INVESTIGATIONS OF SEVEN BITTER MEDICINAL PLANTS OF BANGLADESH" submitted by Md. Tamzid Hossain Molla, M. Phil. Fellow, Department of Applied Chemistry and Chemical Engineering, to the University of Rajshahi, Bangladesh, in partial fulfilment of the requirements for the degree of Master of Philosophy (M. Phil.) is the candidate's own achievement and not a conjoint work with any one else. This is an original work of the author and no part of this thesis has been submitted elsewhere for any degree, diploma or prize. He has fulfilled all the requirements according to the rules of this University. To the best of my knowledge, the results and data presented in this thesis are genuine and original. The author carried out his research under my direct supervision and guidance in the Pharmaceutical Research Laboratory, Department of Applied Chemistry and Chemical Engineering of the same University.

I have gone through the final draft of the thesis and wholehearted recommend its submission for M. Phil. degree.

10/3/2014

Dated, Rajshahi,
March 10, 2014.

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DECLARATION

I do hereby declare that the research work presented in this dissertation entitled "MICROBIOLOGICAL CYTOTOXIC, HAEMATOLOGICAL AND HISTOPATHOLOGICAL INVESTIGATIONS OF SEVEN BITTER MEDICINAL PLANTS OF BANGLADESH" is my own achievement and is not a conjoint work with any one else. This is my original study and no part of this thesis has been submitted to any other University or Institution for any degree, diploma or prize. The thesis contains no materials written or published by any other person except when due reference in made in the text of the thesis. The thesis is submitted to the University of Rajshahi for partial fulfilment of M. Phil. Degree.

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ABSTRACT

Plants are credited with many medicinal properties. Medicinal plants are alleged to possess therapeutic effects in various diseases. Seven bitter medicinal plants of Bangaldesh, viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L. have been selected for present investigation owing to their recognised medicinal importance. The extracts of these plants are used in the treatment of bronchitis, stomachic, carminative, anthelmintic, fever, asthma, inflammation, febrifuge, gonorrhoea, antitumor, liver complaints, vomiting, leprosy, jaudice, dyspepsia, piles, antidiabelic, anti-cancer, leucoderma, lumours, gonorrhoea, rheumatism, diarrhoea, and many other diseases. These extracts are anti-asthmatic, antipasmodic, expectorent, antitussive, antifungal, antimalarial, antibacterial and antimutagenic. Our present work deals with the extraction of the above medicinal plants with ethanol and microbiological, cytotoxic, haematological and histopathological investigations of the extracts of the above plants. The leaves of the above medicinal plants were washed separately with tap-water to remove adhering dirt. They were cut into small pieces with a knife. The chopped pieces were dried in the sun for four days. The sun dried materials were then dried in an electric oven at 40^{o}c for about 72 hours when the leaves became almost dry. These were then removed from the oven and the dried leaves were pulverized into powder and paste with the help of a grinding machine. The whole operations for the leaves of seven medicinal plants were done separately. The powdered and pasty leaves were stored in seven separate air tight containers and kept in a cold, dark place for investigation.

The powdered and pasty leaves were taken in seven separate quick-fit glass stoppered bottles. The contents of the bottles were shaken continuously with ethanol in an electric shaker for about nine hours. The bottles were allowed to stand for several days. The contents of the bottles were filtered successively in a tincture press and the filtrates were separately

collected in seven glass containers. The ethanol extracts of seven medicinal plants were concentrated under reduced pressure when brownish-yellow semi-solid masses were obtained. Each of the concentrated extracts was preserved in a refrigerator for investigations. In every extraction, 250 g of powered or pasty leaves of the plants were taken in 500 ml ethanol. Antimicrobial activity of the ethanol extracts were evaluated by 'Disc diffusion method'[145]. Disc diffusion method is essentially a qualitative or semi quantitative test, which indicates the sensitivity or resistance of microorganism to the test material. The microbiological investigation was done to determine the susceptibility of some pathogenic bacteria to ethanol extracts of the seven medicinal plants under investigation. Dried filter paper discs impregnated separately with the test materials were placed gently on nutrient agar plates inoculated with the test organism. The dried discs absorbed water media and the test materials diffused. As a result there is a gradual change of test materials concentration in the media surrounding each disc. The plates were then incubated at 37.5°C for 24 hours to allow growth of the organisms. If there is any antimicrobial activity of test materials it will inhibit the growth of microorganisms and a clear zone of inhibition will be visualized surrounding the discs. The antimicrobial activity of the test materials is determined by measuring the diameter of zone of inhibition. The larger zone of inhibition is observed for more susceptible organism. The antibacterial activity of the crude ethanol extracts obtained from the leaves of seven medicinal plants (viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L.) were tested against nine bacteria at concentration of 300 μg/disc, 400 μg/disc and 500 μg/disc. Standard antibiotic disc kanamycin (30μg/disc) was used for comparison. The diameters of the zones of inhibition derived by ethanol extracts of the leaves of the plants were measured in mm with a transparent scale and the results were recorded. The minimum inhibitory concentration (MIC) of ethanol extracts of the leaves of the above medicinal plants against four gram-positive

bacteria and five gram-negative bacteria were determined by serial tube dilution technique. The diameters of the zones of inhibition derived by ethanol extract of the leaves of *An. paniculata* showed maximum activity against *B. megaterium* and *Shigella dysenteriae*. No activity of the extract on *Sarcina lutea, Shigella shiga*, *Shigella sonnei* was experienced. The extract of *Vinca rosea* L. was active against *Staphylociccus aureus*, *B. subtilis*, *Sarcina lutea, Salmonella typhi, E. coli* and *Shigella dysenteria*. The extract was found to posses the maximum activity on *B.subtilis* and *Salmonella typhi*. Activity of the extract against *B. megaterium, Shigella shiga* and *Shigella sonnei* was not found. *Ad. vasica* shows the maximum activity against *B. megaterium* and *Shigella dysenteriae*. The extract had no or little activity against *Sarcina lutea*, *Shigella shiga* and *Salmonella typhi*. *V. vegundo* showed the maximum activity on *Staphylociccus aureus* and *Salmonella typhi*. The extract had a little activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*. *A. vera* had the maximum activity against *B. megaterium* and *E. coli*. The extract showed no activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*. *F. ramontchi* showed maximum activity against *B. subtilis* and *Shigella sonnei*. The activity of the extract against *Sarcina lutea*, *Shigella shiga* and *Salmonella typhi* was not observed. *N. arbortristis* was very active against *B. subtilis* and *Salmonella typhi*. The activity of the extract against *B. megaterium*, *Sarcina lutea* and *Shigella shiga* was not found.

The Minimum Inhibitory Concentration (MIC) of the crude ethanol extract was determined by Serial Tube Dilution Technique against nine test organisms mentioned earlier. Nutrient broth medium and culture media were prepared earlier following the standard methods[143,145]. Fresh culture of the pathogenic microorganisms, preparation of test samples, placement of the discs and incubation were achieved accordingly. Kanamycin (30 μg/disc) was used as standards disc. Crude ethanol extracts of the leaves of the above medicinal plants were transferred in seven separate vials containing 2% DMSO solution (2ml). This was mixed well to achieve sample solutions having concentration 1024 μg/ml. Nine sterilized test tubes

containing 1 μg/ml, 2 μg/ml, 4μg/ml, 8μg/ml, 16μg/ml, 32μg/ml, 64μg/ml, 128μg/ml and 256μg/ml sample solutions were prepared for each of the 7 ethanol extracts by SDT[148]. Nine test tubes containing ethanol extracts of *Andrographis paniculata* N. having the above concentrations were considered for determining the MIC against *Bacillus megaterium*. Three test tubes containing media (C_M), media plus sample (C_{MS}) and media plus inoculums (C_{MI}) were also maintained for the experiments. Diluted inoculums (10μl) was added to each of the nine test tubes and mixed well. One ml of the sample was added to C_{MS} and mixed well. 10μl of inoculums of *Bacillus megaterium* was added to C_{MI} to observe the growth of the organisms in the media. C_M containing media was used the check the sterility of the solution. Twelve test tubes were incubated at 37.5^oC for 24 hours. After incubation, the test tubes were examined for visible growth of the organisms. The lowest concentration of the test material at which there was no visible growth was recorded as the MIC of the test material for the test organisms. The first sign of inhibition of test organisms by the ethanol extract of *Andrographis paniculata* N. was visualized in the test tubes and recorded. Similar 8 experiments were performed for the same extract against remaining 8 bacteria and MIC of the particular extract against above nine bacteria were recorded.

Same experimental procedures were followed for the determination of MIC of remaining 6 extracts (viz, extracts of *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L.) against nine bacteria mentioned above. Thus the MIC of seven ethanol extracts of the leaves of above seven plants against nine bacterial strains were determined and the results were recorded in 63 tables. Thus the MIC values of ethanol extracts of the leaves of the above medicinal plants were determined. It was evident from the results that all the extracts had notable antimicrobial activities against most of the test organisms. The extract of the plants had MIC values ranging from 32 to 128 mg/ml. All plant extracts showed no MIC against *Shigella shiga* only. These extracts had notable activities on other bacterial strains mentioned above.

Appreciable MIC of the extracts has been enunciated in the results. The first sign of inhibition of organisms was seen in the test tube against the respective extracts having lower concentrations than those cited in the results had no inhibitory activities on the organisms. The results revealed that the ethanol extracts of the plants under present investigation possess appreciable antibacterial activities. The MIC values of the extracts have remarkable significance about the therapeutic effects of the active principles associated with the leaves of the plants.

Cytotoxic effects of ethanol extracts of the plants were investigated. Brine Shrimp Lethality bioassay[150] indicates cytotoxicity and a wide range of pharmacological activities of the compounds as well. It utilizes a large number of organisms for statistical validation and a relatively small amount of sample. Generally the median effective dose (ED_{50}) values for cytotoxicities are one tenth of median Lethal Concentration (LC_{50}) values in the Brine Shrimp bioassay.[151-155] This can be accompanied by testing in vitro lethality in a simple zoologic organism, viz, Brine Shrimp nauplii, Leach (Artemia Salina). In this method, bioactivity can be found not only for natural product extracts but also for pure compounds. Brine Shrimp Lethality test is a positive correlation between Brine Shrimp toxicity and cytotoxicity. The crude ethanol extracts of seven medicinal plants under present investigation represented positive response to Brine Shrimp Lethality Bioassay. Lethal concentration (LC_{50}) of Brine Shrimp Lethality was determined to evaluate the cytotoxic effect of ethanol extracts. LC_{50} of Brine Shrimp Lethality was obtained from a plot of percentage of mortality versus concentration of the extracts on the graph. The results, presented in the tables and graphs, established a positive correlation between Brine Shrimp Lethality and cytotoxicity. Brine Shrimp Lethality bioassay resembled that the ethanol extract the leaves of *Andrographis paniculata* N. is more cytotoxic (LC_{50}= 30 μg/ml) than those of other extracts. The very low LC_{50} indicated the high toxic effect of the extract of the leaves of the plant. Low cytotoxic effect of each of *Vitex vegundo* L. and *Nyctanthes arbortristis* L. (LC_{50}= 72 μg/ml) was observed. The other ethanol extracts exhibited moderate cytotoxic effects. Thus, it may be conclude that the

ethanol extracts of the leaves of seven medicinal plants under present investigation have cytotoxic effects.

Haematological investigation deals with the composition , formation , destruction, production and conservation of blood and related substances in blood , blood plasma and tissue fluids. Hence, the dietary effects of ethanol extracts of the medicinal plants under present investigation supplemented separately with formulated cereal at 5% level have been investigated in the present study. A survey of 28 consecutive days on young male albino rats, body weight ranging 52-56 g was performed. The dietary effects of the extracts at 5% level with formulated cereal on the composition of red cells like erythrocytes and white cells, viz, leucocytes and platelets (thrombocytes) were investigated in this experiment. Its effects on the composition of white cells like esnophil, neutrophil, monocytes, basophil and lymphocytes were investigated. Haematological parameters, i,e, haemoglobin, red blood cells (RBC), erythrocyte sedimentation rate (ESR), cholesterol, white blood cells (WBC) and total count of WBC of the rats under experimental and control groups were investigated after feeding the extracts, separately fortified with the formulated cereal at 5% level for a period of 28 consecutive days.

Histopathological as well as toxicological effects of the ethanol extracts were investigated on livers, lungs, kidneys, hearts and spleens of the experimental rats. These were achieved by observing the changes in the cellular structures of these organs. The present investigation included the identification and toxicological effects in those organs of the experimental rats. These were achieved by observing any changes in the cellular structures (degradation and regeneration) of organs of the experiment rats after taking fortified cereal for a period of 28 consecutive days. Dietary effects of feeding on the experimental rats during the experimental period were evaluated by careful observation of the depression of central nervous system, excitation, muscular reflexes and behaviour of the rats.

Haematological and histopathological investigations of the ethanol extracts of the plants supplemented separately with formulated cereal at 5% level on young male albino rats after feeding for 28 consecutive days both for the experimental rats and control group were performed. During this experiment, the effect of feeding the supplemented cereal on gain in body weights of the rats during the experimental period was investigated simultaneously. A control group of the rats for formulated cereal (without having any extracts) was also maintained for gain in body weight. Initial body weight of the rats, gain in body weight during the experiment of feeding and gain in body weight per one gram of food intake by each of the rats were recorded. The results resembled that there was a close similarity of the diets in gain in body weight of the rats. It appeared from the results that the diets had almost equal impact in gain in body weight. The results were computed to show the gain in body weight for one gram of food intake by 16 young male albino rats for feeding eight diets. Analysis of variance table and a difference table for multiple comparison of mean gain in body weight per one gram of food intake were computed to show which of the diets was statistically more significant for gain in body weight and for the normal development of the rats. It was evident from the tables that all the diets had similar impacts for normal development and maintenance of the rats. None of the diets was statistically significant in gain in body weight of the rats. Separate tables were prepared for Dancan's New Multiple Range Test for protection level $\alpha = 0.01$ and $\alpha = 0.05$. The tables failed to identify which of the diets was statistically significant at 1% or 5% levels of significance. The tables also showed that the treatments and errors of the diets had no Least Significance Range (LSR) in comparison with their respective Standard Significance Range (SSR).[162]

Total count of RBC and WBC, differential count of WBC and percentage of haemoglobin in the blood of the rats were investigated and the results were summarized. Comparing these values with those of reference values for haematological parameters it resembled that the changes in the parameters remained within the normal limit. No abnormalities in

haematological investigations were detected. During the experimental period, the rats under investigation were found to restore no signs of muscular numbness of the legs, salivation, excitation, weakness, diarrhoea, etc. and no reflex abnormalities were detected. Thus it appeared that the dietary effects of feeding the ethanol extracts of the plants fortified at 5% level with formulated cereal for a period of 28 consecutive days had no adverse action on both control and experimental rats.

At the end of the feeding experiment for a period of 28 consecutive days, the rats were sacrificed under mild anaesthesia with ether-chloroform and their livers, lungs, kidneys, hearts and spleens were preserved, processed, sliced and the tissues were mounted on glass slides and viewed under microscope to find any histopathological change in the organ. Microscopic view of sliced tissues of the organs on glass slides were recorded. On gross necropsy evaluation, all the organs under examination exhibited normal colour. No abnormal histopathological change in the cellular structure in the tissues was detected. This implied from microscopic view of those organs that the effect of feeding the ethanol extract at 5% level with the formulated cereal for 28 consecutive days had no adverse effect on cellular structure of those organs of the experiment rats and no morphological changes occurred therein. From the foregoing evidences, it appeared that the ethanol extracts of the above plants, fortified with formulated cereal at 5% level was non-toxic.

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Chapter I

Introduction

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

Chapter I

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources. Plants are being used by human beings from time immemorial. Human civilization and other living organisms are to depend to a great extent on plants, its roots, stems, leaves, barks, wood, fruit, oils, herbs, shrubs, bushes etc. Plants are unique in their ability to synthesis carbohydrates, fats and proteins which constitute three major food classes for human race. Apart from these, certain other substances such as glycosides, alkaloids, vitamins, saponins, essential oils, resins, tannins and bitter principles etc. are produced within the plants by their metabolic activities. Some of these, in regulated doses, are effective remedies for many diseases. Plants containing medicinal principles are usually classified as medicinal plants.

Medicinal plants are alleged to possess therapeutic effects in various diseases. From medicinal point of view, the great importance of active principles from medicinal plants are specially notable. The science of medicine is very old. Plants and plants products have been used to alleviate the sufferings of the body and mind from time immemorial. The earliest records of therapeutic use of plants came from the ancient civilizations of the Chinese, the Hindus, the Greeks and the Mediterranean peoples of antiquity. The ancient Egyptians[1] knew the use of substances of plant origin and had an idea of many basic forms of drug mixture (gargles, ointments, poultices). The Romans introduced the empirical methods of collection and therapeutic administration of herbs and herbal preparations. Theophrastus[2] (370-285 B.C), a student of Plato, in his Historia Plantarum described the uses of nearly 500 medicinal plants. The South American natives knew the

paralytic effects of Curare and used it as arrow poison. Ipecacuanha[3] root was used in Brazil and Far East for dysentery and diarrhoea. The antileprotic effect of Chulmoogra fruit was known to the ancient Indians. The medicine men of the Hebrews and the Mexicans knew the use of Chenopodium Anthelminticum[3] as worn expellant. Chincona bark was used by the South American Indian as a fabrifuge.

The discovery of the medicinal plants of Indian Subcontinent was made hundreds of years ago. Rigveda[4], Atharvaveda, Ayurveda, Susruta and Charaka[5] are amongst the oldest treatises on Indian medicinal plants. In Indian literatures, many ancient plants have been described, of which the use of Croton as a purgative; the smoking of Datura in case of asthma and the use of Nuxvomica in paralysis and dyspepsia are important. During the middle ages when the Romanian Glory were declining, the Arabs contributed much to the vast field of medicine. Canons of Medicine[6] by Ibn Sina bears testimony to this. With the advent of the Muslim conquerors, the Mohammadan sysem of medicine was introduced in the subcontinent and the system was prevalent during the region of Mogal and Pathan. The Arabian system of medicine was of superior standing and was fairly advanced for the period. An excellent account of the medicinal plants of the Indian Subcontinent can be seen in the book written by Abu Mansur in that period.

Although the importance of investigation was realized by early workers and the therapeutic properties of various plants product have been known and utilized from early it was not until 1803, that the first physiologically active principle isolated, was a crude form of the alkaloid Narcotine when Derosne[7] obtained from a syrupy extract of opium. This beginning opened up an era of successful isolation of a large number of other physiologically active principles of plant origin. Morphine was isolated from opium by Sertuner[8] in 1806, quinine by Pelletier and Caventou[9] in 1820, and the mydriatic base Atropine by Mein, Geiger and Hesse[10] in 1833. In 1864, physostignine was isolated from Calabarbean by Jabst and Hess[11]. This was followed by the isolation of the anthelmintic alkaloid Pelletierine[12] from pomegranate in 1877 and of Ephedrine[13] from

Ephedra vulgaris in 1887. Many other chemical compounds have since then been isolated and their chemistry and pharmacological action studied.

In spite of these, interests[13-17] in the chemistry and pharmacology of medicinal plants have increased considerably in recent years. Although researches in medicinal plants may not always produces new and potent drugs, the background knowledge regarding the chemical structure and pharmacological action which such work gives may open up newer possibilities for creative synthesis.

Nature has endowed Bangladesh with enormous plant resources. Many of these plants grow wild in jungles, forests, gardens and many of them are found lying everywhere in the fertile region of the country. Some of these plants are used in Ayurvedic and Hekimi system of medicine prevalent in the country. Of the large number of plants used in indigenous system of medicine, there are many that deserve the reputation, they have earned as cures while others may have little or no effects. Studies of some of these plants in the recent past have been much rewarding. Extensive researches have been done in Bangladesh on *Rauwlfia Serpentina*[18,19], *Andrographis peniculata* Nees (B. Kalmegh)[20,21], *Cephalandra Indiea* Naud (B. Tela-Kucha)[22,23], *Datura fastuosa* Linn[24], *Abrus precatrius* Linn (B. Kunch)[25,26,27], *Terninalia arjuna* Bedd (B. Arjun tree)[28], *Eupatorum odoratum* Linn (B. Assan Lata)[29], *Scoparia dulcis* Linn,[30], *Pimpinelia anisum* (B. Souf)[31], *Cassia fistula* Linn (B. Sonari)[32], *Leucas aspera* Spreng (B. dandakalash or ghalghase)[33], etc.

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well being. In our country we are using crude plants as medicine since Vedic period. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources.[34]

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization.[35] Although a significant number of studies have been used to obtain purified

phytochemicals, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants.[36] The greater susceptibility of gram-positive bacteria to plant extracts has been previously reported in South American,[37] African[38,39] and Australian[40] medicinal plant extracts. Susceptibility differences between gram-positive and gram-negative bacteria may be due to cell wall structural differences between these classes of bacteria. The gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics[41]. *C. roseus* showed MIC 128 mg/ml[42] evaluated non-polar (dichloromethane) and polar (MeOH and aqueous) extracts of *An. paniculata* (whole plant) for *in vitro* antibacterial activity against 12 skin diseases causing bacterial strains using the disc diffusion method at three concentrations; 1000, 500, and 250 µg/disc respectively and found significant antibacterial activities against both the Gram-positive and Gram-negative bacterial strains tested. Diterpenoids and flavonoids are the main chemical constituents of *An. paniculata* which are believed to be responsible for the most biological activities of this plant.[43] *C. roseus* has a variety of medicinal properties, such as antibacterial,[44] antifungal[45] and antiviral.[46] The alkaloids from *C. roseus* are famous for their anticancer activity[47,48]. Studies revealed its wound healing action in the rats.[49] One of the isolated endophytes produced potential antimicrobial activity against some selected human pathogenic bacteria and a yeast.[50]

The previous results of the phytochemical analysis of *Ad. vasica* show that phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars are present in the leaves. It has also been shown that tannins are biologically active, against *E. coli Staphylococcus aureus*, *Salmonila paratyphi* and *Candida albicans*.[51] The Preliminary phytochemical screening of successive extracts indicated presence of lipids, flavonoids, saponins, alkaloids, tannins, carbohydrates, terpenoids, and steroids in *F. ramontchi* leaves.[52] Antimicrobial susceptibility test showed that both the gel and the leaf of *Al. vera* inhibited the growth of *S. aureus*.[53] Methanol extract of *F. ramontchi* leaves possess broad-spectrum

antimicrobial activity at concentration 10000 µg/ml whereas hydromethanolic and chloroform extracts having more or less antimicrobial activity.[54] Leaves extracts of *N. arbortristis* was found to have antimicrobial activity.[55] The chloroform extract of this plant was found to have both antibacterial and antifungal activity whereas the petroleum ether and ethanol extracts possess only antibacterial activity .[56,57]

The works cited above covers only a very small portion of the vast number of plants that are used in indigenous medicine and a great majority of these plants remains yet to be investigated. Re-emphasized researches in plants for medicinal agents and researches in indigenous medicinal plants, seeds and medicinal seed oils are in progress in different laboratories of the country.

Introduction to the plants under present investigation :

Plants are credited with many medicinal properties. Extracts of the following medicinal plants viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L. under present investigations are used in the treatment of dyspepsia, flatulence, hemorrhoids, constipation, chronic diarrhoea and dysentery, hepatosplenomegaly, intestinal parasites, cholelithiasis, fever, sore throat, pharyngitis, laryngitis, cough, catarrh, bronchitis, skin diseases, edema, ophthalmia, alopecia, premature graying, headache, dropsy, piles, expectorant and laxative. Plants extracts are anti-asthmatic, antipasmodic, expectorent, antitussive, antifungal, antimalarial, antibacterial and antimutagenic.[58,59]

In spite of having the reported usefulness of the extracts of these plants in folk-medicine, no such works on their microbiological, cytotoxic, haematological and histopathological investigations have so far been reported in the literature.

Our present investigation deals with the extraction of the above medicinal plants with ethanol and microbiological, cytotoxic, haematological and histopathological investigations of the extracts of the above plants.

Literature review and morphology of the plants:

***Andrographis paniculata* N. :**

Andrographis paniculata N. is a herbaceous plant in the family *Acanthaceae*, native to India and Bangladesh. *Andrographis paniculata* N. is an erect annual herb extremely bitter in taste in all parts of the plant body, thus it is commonly known as king of bitter. As an Ayurveda herb it is known as *Kalmegh* or *Kalamegha*. The blood purifying property of this plant results in its use in diseases where blood abnormalities are believed the causes of disease, such as skin eruptions, boils, scabies, and chronic undetermined fevers. The aerial part of the plant used medicinally, contains a large number of chemical constituents, mainly lactones, diterpenoids, diterpene, glycosides, flavonoids, and flavonoid glycosides[60].

Habitat and morphology description of *Andrographis paniculata* N. :

Andrographis paniculata N. grows erect to a height of 30-110 cm in moist shady places with glabrous leaves and white flowers with rose-purple spots on the petals. The stem dark green, 0.3-1.0 m in height, 2-6mm in diameter, quadrangular with longitudinal furrows and wings in the angles of the younger parts, slightly enlarged at the nodes; leaves glabrous, up to 8.0 cm long and 2.5 cm broad, lanceolate, pinnate; flowers small, in lax spreading axillary and terminal racemes or panicles; capsules linear-oblong, acute at both ends, 1.9cm × .03 cm; seeds numerous, dub quadrate, yellowish brown (Fig. 1)[61]. it grows abundantly in south-eastern Asia, i.e., India, Bangladesh, Sri Lanka, Pakistan, Java, Malaysia and Indonesia, while it is cultivated extensively in India, China and Thailand.[62]

Distribution:

A. paniculata is distributed in tropical Asian countries, often in isolated patches. It can be found in a variety of habitats, such as plains, hillsides, coastlines, and disturbed and cultivated areas such as roadsides, farms, and wastelands. Native populations of *A. paniculata* are spread throughout south India and Sri Lanka which perhaps represent the center of

origin and diversity of the species. The herb is an introduced species in northern parts of India, Java, Malaysia, Indonesia, the West Indies, and elsewhere in the United States of America ***Andrographis paniculata* N.** The species also occurs in Hong Kong, Thailand, Brunei, Singapore, and other parts of Asia where it may or may not be native. The plant is cultivated in many areas as well. Unlike other species of the genus, *A. paniculata* is of common occurrence in most places in India, including the plains and hilly areas up to 500 m, which accounts for its wide use. Since time immemorial, village and ethnic communities in India have been using this herb for treating a variety of ailments.[63]

Botanical information of of *Andrographis paniculata* N. :[64]

Scientific classification

Kingdom:	Planeta
Division:	Angiosperms
Class:	Eudicots
Order:	Lamiales
Family:	*Acanthaceae*
Genus:	*Andrographis*
Species:	*A. paniculata*

Vernacular Name:

Bengali	Kalmegh
English	Green chirayta, creat, king of bitters, andrographis
Sanskrit	Kalamegha, Bhunimba
Hindi	Kirayat
Oriya	(Bhuinimba)
Tamil	Nilavembu, Sirunangai, Siriyanangai
Malay	Hempedu Bumi
Thai	Fa Thalai Chon ,
Chinese	Chuan Xin Lian
Bahasa Indonesia	Sambiloto
Arabic	Quasabhuva
Kannada	Nelabevu

Medicinal importance and uses of *Andrographis paniculata* N.:

A. peniculata is used in traditional medicine in India, Bangladesh and some other countries for multiple clinical applications. From a biomedicinal perspective, the therapeutic value of Kalmegh is due to its mechanism of action which is perhaps by enzyme induction. The plant extract exhibits antityphoid and antifungal activities. Kalmegh is also reported to possess antihepatotoxic, antibiotic, antimalarial, antihepatitic, antithrombogenic, antiinflammatory,[65] anti-snake venom, and antipyretic properties. Beside these usefulness, the plant is well known for its general use as an immunostimulant agent.[66] A study conducted at Bastyr University, showed a significant rise in the mean CD4 lymphocyte level of HIV subjects after administration of 10 mg/kg andrographolide,[67] the chief constituent extracted from the leaves of the plant. The herb has shown an ability to reduce inflammation (heat) and fight viral infection, and is used as a principal ingredient in traditional Chinese medicinal formulas for lung support from colds.[68] In one Chilean study, the herb had a significant drying

effect on the nasal secretions of cold sufferers who took 1,200 milligrams of andrographis extract daily for five days.[69] A systematic review of the literature and meta-analysis of randomized controlled trials also suggested the herb alone or in combination with eleuthero may be an appropriate alternative treatment of uncomplicated acute upper respiratory tract infection.,[70,71] A recent randomised, double-blind, multicentre study found *Andrographis paniculata* N. as effective as mesalazine (mesalamine) in ulcerative colitis.[72] Further, andrographolide inhibits interleukin-6 expression and suppresses prostate cancer cell growth *in vitro*.[73] Andrographis has been shown to be a safe traditional botanical for supporting upper respiratory tract health, per analysis of seven double-blind controlled trials.[74] The herb has been shown to inhibit RANTES secretion in inflamed bronchial cells.[75] RANTES is a chemoattractant for eosinophils, monocytes and lymphocytes that is stored in, and released by, platelets and activated T-cells. In related research: Andrographolide, an active ingredient in Andrographis, has been shown to be responsible for the herb's inflammatory modulating actions, including the reduction of cytokine and peritoneal deposition of neutrophils, and modulation of lung inflammation in vivo.[76] Extracts of Andrographis exhibit potent inflammatory modulating and antioxidant actions in mouse models.[77] Administration of *Andrographis paniculata* N. prevented hexachlorocyclohexane induced activities of γ-glutamyl transpeptidase, glutathione-S-transferase and lipid peroxidation in mouse liver. An indication of potential antioxidant and hepatoprotective effects of *Andrographis paniculata* N.[78] were observed. The plant has been reviewed to be superior to placebo in alleviating the subjective symptoms of uncomplicated upper respiratory tract infection (URI) and being preliminary evidence of a prevalence effect.[79]

***Vinca rosea* Linn. :**

Vinca rosea L. is a perennial herb with slender tubular flowers. It is common on sandy tropical beaches and popular in tropical gardens as it flowers continuously. Sometimes it can be found growing rooted in cracks in old buildings. The leaves are always placed in pairs on the stem, the flowers springing from their axils. It propagates itself by long, trailing and rooting stem. The flowers vary somewhat in intensity of color, but horticulturist have developed varieties with colors ranging from white to hot pink to purple.[80]

Habitat and morphology description of *Vinca rosea* L. :

It is an evergreen sub shrub or herbaceous plant growing to 1 m tall. The leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1–1.8 cm long; they are arranged in opposite pairs. The flowers are white to dark pink with a darker red centre, with a basal tube 2.5–3 cm long and a corolla 2–5 cm diameter with five petal-like lobes. The fruit is a pair of follicles 2–4 cm long and 3 mm broad.[81,82,83,84]

Distribution:

The plant has naturalized in much of the tropical world, as well as all parts of Bangladesh. The plant may be grown as a perennial in our country. It can grow in a full-sun or partially shaded location. Fertile soils will actually harm the plant, and result in reduced flowering. The plant prefers to be somewhat dry, requiring only the occasional watering during the growing season. Over watering can quickly lead to rot diseases, which will kill the plant.

Botanical information of *Vinca rosea* L.:[64,84]

Scientific classification

Kingdom:	Plantae
Division:	Angiosperms
Class:	Eudicots
Order:	Gentianales
Family:	*Apocynaceae*
Genus:	*Catharanthus*
Species:	*C. roseus*

Vernacular Name:

Sanskrit	Nityakalyani
Bengali	Nayantara
Hindi	Sadabahar
Malayalam	Shavam Naari
Marathi	Sadaphuli
Gujarati	Baarmaasi
Punjabi	Rattanjot
Uriya	Ainskati
Tamil	Nityhakalyani

Medicinal importance and uses of *Vinca rosea* L.:

Vinca rosea is used in medicine for their acrid, astringent and tonic properties.[80] Its traditional value used as a treatment for diabetes has led to extensive investigation into its properties. Numerous animal studies have shown that ethanolic extracts of leaves of *Vinca rosea* lower blood glucose levels.[85] More recently, extracts from *Vinca rosea* have been shown to be effective in the treatment of various kinds of leukemia, skin cancer, lymph cancer, and Hodgkin's disease.[86,87] In Jamaica, an extract of the flowers was used to make a solution to treat eye irritation and infections. In India, the juice from the leaves was treated to wasp sting. Besides that, an extract of the boiled plant was used to arrest bleeding and make a gargle to ease sore throats.[88] *Vinca rosea* is reported to have analgesic, local anesthetic and anti-inflammatory properties. It contains over 70 different indole alkaloids. Two of these, vincristine and vinblastine are powerful anticancer agents which inhibit the growth of tumors and are two of the most important medicinal compounds found in plants in the last 40 years.[88] Hot water extract of leaves is taken orally by pregnant woman to combat primary inertia in childbirth and the boiled leaves are drink to treat diabetes.[89] Hot water extract of leaves is taken orally for diabetes and rheumatism and the root extract is taken orally as hypotensive and febrifuge.[90] The dried entire plant is taken orally by human adults for cancers, heart disease and leishmaniasis.[91] Decoction of dried entire plant is used orally by human adults to treat liver disease.[92]

***Adhatoda vasica* Nees. :**

Adhatoda vasica N. is a species of plant in the *Acanthaceae* family. It is a well-known herb in indigenous systems of medicine for its beneficial effects, particularly in bronchitis. It is tall, with several branches, dense, and an evergreen shrub. Acknowledging its medicinal properties, it has been adopted by modern medical practitioners. It has been used extensively as an important herbal drug in treating a wide variety of diseases and the leaves of the plant are the main source of drug formulation. Vasaka leaves, bark, the root bark, he fruit and flowers are useful in the removal of intestinal parasites. Vasaka herb is used for treating cold, cough, chronic bronchitis and asthma.[93]

Habitat and morphology description of *Adhatoda vasica* N. :

This is a shrub with lance-shaped leaves 10 to 15 centimeters in length by four wide. They are oppositely arranged, smooth-edged, and borne on short petioles. When dry they are of a dull brownish-green colour. They taste bitter. When a leaf is cleared with chloral hydrate and examined microscopically the oval stomata can be seen. They are surrounded by two crescent-shaped cells at right angles to the ostiole. The epidermis bears simple one- to three-celled warty hairs, and small glandular hairs. Cystoliths occur beneath the epidermis of the underside of the blade.[94]

Distribution:

This plant occurs throughout Bangladesh except mountain regions. Leaves are long and dark green in colour. Cattle do not eat this plant as the leaves emit an unpleasant smell.

Botanical information of *Adhatoda vasica* N. :[95]

Scientific classification

Kingdom:	Planeta
Division:	Angiosperms
Class:	Eudicots
Order:	Lamiales
Family:	*Acanthaceae*
Genus:	*Justicia*
Species:	*J. adhatoda*

Vernacular Name:

Sanskrit	Sinhapuri, Vasaka
Bengali	Adulsa, Bakash,Vasok
Hindi	Adosa, Arusha, Rus, Bansa
Malayalam	Atalotakam
Marathi	Adulsa, Adusa
Gujarati	Adulso, Aduraspee, Bansa
Persian	Bansa
Kannada	Adusogae
Tamil	Adathodai

Medicinal importance and uses of *Adhatoda vasica* N. :

The plant *Adhatoda vasica* N. has potent antiperiodic, astringent, diuretic and purgative action. It is a highly valued Bangladeshi medicinal plant, which is used in the treatment of respiratory diseases like asthma, cough, bronchitis and tuberculosis. The flowers, leaves and root have antispasmodic property. The activities against tuberculosis were reported by many researchers quite early.[95] For instance, the source of the drug 'vasaka' is well known in the indigenous system of medicine for its beneficial health effects, particularly in treating bronchitis.[96] Traditionally, *Adhatoda vasica* N. has been used for the treatment of respiratory disorders namely acute and chronic cough, bronchitis and asthma. It is also used as an expectorant in the treatment of acute and chronic bronchial catarrh and pulmonary diseases. The leaves, as well as, the flowers, fruits and roots are extensively used for the treatment of cold, whooping cough, asthma and as antihelmintic and the leaf extract is known to cure diarrhoea, dysentery and glandular tumor. The different parts of the plant is used in the Bangladeshi traditional medicine for the treatment of various diseases like asthma, joint pain, lumber pain and sprains, cough, eczema, malaria, rheumatism, swellings, venereal diseases.[97] In homeopathy, *Adhatoda vasica* N. has been used in the treatment of cold, cough, pneumonia, spitting of blood, fever, jaundice, catarrh, whooping cough and asthma.[98]

***Vitex vegundo* L. :**

Vitex vegundo L. is an aromatic large shrub or small slender tree of about 3 meter in height with quadrangular branches. It is found in moist area, often on banks of rivers, throughout Bangladesh. The plant has pungent, bitter, acrid taste; heating, astringent, cephalic, stomachic, anthelmintic properties. Various medicinal properties are attributed to it particularly in the treatment of anti-inflammatory, fungal diseases, antioxidant and hepatoprotective disorders.[99,100]

Habitat and morphology description of *Vitex vegundo* L.:

A large shrub or sometimes a small slender tree; bark thin, grey; branchlets uadrangular, whitish with a fine tomentum. Leaves 3-5 foliate; leaflets lancoelate, acute, the terminal leaflet 5-10 by 1.6-3.2 cm. with a petiole 1-1.3 cm. long, the lateral leaflets smaller with a very short petiole, all nearly glaborous above, covered with a fine white tomentum beneath, base acute; common petioles2.5-3.8 cm long.[101,102,103] Flowers in pedunculate branched tomentose cymes, opposite along the quadrangular tomentose rachis of a large terminal often compound pyramidal panicle (axillary peduncles in the upper axils sometimes present); bracts 1.5- 2.5 mm long, lancoelate caduceus. Calyx 3 mm long, white tomentose; teeth triangular, 0.8-1mm long. Ovary glaborous; style glaborous; stigma forked. Drupe less than 6 mm diameter, black when ripe.[99,101,102,103]

Distribution:

Vitex vegundo L. is widely planted as a hedge plant along roads and between fields. Found throughout the greater part of Bangladesh, often occurring gregariously; it is abundant along river banks, in moist situations, open wastelands and near deciduous forests.

Botanical information of *Vitex vegundo* L.:[64]

Scientific classification

Kingdom:	Planeta
Division:	Angiosperms
Class:	Eudicots
Order:	Lamiales
Family:	*Lamiaceae*
Genus:	*Vitex*
Species:	*V. negundo*

Vernacular Name:[101]

Sanskrit	Nirgundi; Sindhuvara;
Bengali	Nishinda
Hindi	Nirgundi; Sambhalu;
Malayalam	Indrani
Marathi	Nirgunda
Gujarati	Nagoda; Shamalic
Chinese	Huang jing
Kannada	Bile-nekki
Tamil	Chinduvaram; Nirnochchi

Medicinal importance and uses of *Vitex vegundo* L.:

The plant has pungent, bitter, acrid taste; heating, astringent, stomachic, anthelmintic; promotes the growth of hair; useful in disease of the eye, consumption, inflammation, leucoderma, enlargement of the spleen, bronchitis, asthma, biliousness, painful teething of children. The root is an antidote to snake venom. The root is considered tonic, febrifuge and expectorant,[99,102104] otalgia, arthritis, dyspepsia, colic, rheumatism, leprosy, verminosis, flatulence, dysentery, urinary disorders, wounds, ulcers, bronchitis, cough, malarial fever, haemorrhoids, dysmenorrhoea,leprosy, skin diseases and general debility. The plant is reported to have expectorant, carminative, digestive, anodyne, antiseptic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic.[102] The leaves are aromatic, tonic and vermifuge.[101,104] A decoction of Nirgundi leaves is given with the addition of long pepper in catarrhal fever with heaviness of head and dullness of hearing. A pillow stuffed with the leaves of Nirgundi is placed underthe head for relief of headache. The juice of the leaves is said to have the property of removing foetid discharges and worms from ulcers.[99,101,104] The flowers are useful in diarrhoea, cholera, fever, haemorrhages, hepatopathy and cardiac disorders. Leaves and bark are useful in scorpion stings, seeds are considered useful in eyediseases in form of anjan.[102] Tincture of root bark in 1 to 2 dr. doses is recommended in cases of irritable bladder and of rheumatism. Powdered root is prescribed for piles as a demulcent for dysentery. Root is used indyspepsia, colic, rheumatism, worms, boils and leprosy.[99,104] The leaves are discutient and are useful in dispersing swelling of joints from acute rheumatism and of the testes fromsuppressed gonorrhoea. The dried fruit acts as a vermifuge.[101,104] Fruit is nervine, cephalic and emmenagogue; dried fruits acts as a vermifuge; flowers are cool and astringent.[104]

***Aloe indica* W. (*Aloe vera*) :**

The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name *Aloe vera* derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." 2000 years ago, the Greek scientists regarded *Aloe vera* as the universal panacea. The Egyptians called Aloe "the plant of immortality." Today, the ` plant has been used for various purposes in dermatology.[105]

Habitat and morphology description of *Aloe indica* W. :

The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. Each leaf is composed of three layers: 1) An inner clear gel that contains 99% water and rest is made of glucomannans, amino acids, lipids, sterols and vitamins. 2) The middle layer of latex which is the bitter yellow sap and contains anthraquinones and glycosides. 3) The outer thick layer of 15–20 cells called as rind which has protective function and synthesizes carbohydrates and proteins. Inside the rind are vascular bundles responsible for transportation of substances such as water (xylem) and starch (phloem).[106]

Distribution:

Aloe vera is a perennial, succulent plant with fleshy leaves. It is a native of North Africa. Now the plant is much more widespread and can be found growing throughout Europe and North America as well as South America, the Middle East, China, Bangladesh, India, Pakistan and Australia. The plant is distributed throughout the India. It has become completely naturalized, especially in the hot dry valley of northwestern Himalaya. It is planted as hedge in house premises, runs wild in desert conditions and is also extensively cultivated due to its medicinal properties. The *Aloe vera* habitat needs direct sunrays and well-drained soil. When these plants are grown outdoors, they need the warmth of sunrays and protection from the cool weather because the plant consists of 95% water.[107]

Botanical information of *Aloe indica* W. :[108]

Scientific classification

Kingdom:	Planeta
Division:	Magnoliophyta
Class:	Liliopsida
Order:	Liliales
Family:	Aloacae
Genus:	*Aloe*
Species:	*Aloe vera*

Vernacular Name:

Sanskrit	Kumari
Bengali	Ghritakumari
Hindi	Gheekumari
Malayalam	Chotthu kathalai
Marathi	Khorpad
German	Echte Aloe
Chinese	Lu Hui
Tamil	Kathalai

Medicinal importance and uses of *Aloe indica* W. :

Aloe vera gel has been reported to have a protective effect against radiation damage to the skin.[109,110] Exact role is not known, but following the administration of *Aloe vera* gel, an antioxidant protein, metallothionein, is generated in the skin, which scavenges hydroxyl radicals and prevents suppression of superoxide dismutase and glutathione peroxidase in the skin. It reduces the production and release of skin keratinocyte-derived immunosuppressive cytokines such as interleukin-10 (IL-10) and hence prevents UV-induced suppression of delayed type hypersensitivity.[111] *Aloe vera* inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts.[112] Polysaccharides in the *Aloe vera* plant have curative effects on numerous digestive disorders. *Aloe vera* cure IBS, ulcers, Crohn's disease, ulcerative colitis[113,114] and other disorders of the digestive tract. This is one of the best-known applications of *Aloe vera* gel.[115] *Aloe vera* ends acid reflux disease: Taking *Aloe vera* gel on a regular basis, reduce your heartburn symptoms within a few days.[116] Dried latex from the inner lining of aloe leaves has been used traditionally as a laxative taken orally. So combination herbal remedy containing aloe was found to be an effective laxative in research.[117] Pain in the joints and muscle pain occurred due to arthritis is reduced by the application of *Aloe vera* sprays or gels.[117] Using aloe topically is well known to ease inflammation of joints, reducing arthritis pain. But *Aloe vera* can also be used internally, reducing inflammation throughout the body from the inside out. People who drink *Aloe vera* for two weeks typically begin to experience a significant reduction of inflammation symptoms. It protects the kidneys from kidney infection and urinary tract infection. It prevents kidney stones and protects the body from oxalates in coffee and tea.[117] Laboratory studies show that *Aloe vera* can stimulate insulin release from the pancreas and can lower blood glucose levels in mice. Results from two poorly conducted human trials suggest that oral *Aloe vera* gel may be effective in lowering blood glucose levels.[118]

***Flacourtia ramontchi* :**

Flacourtia indica (Burm. f.) Merr. Synonymous to *Flacourtia ramontchi* L'Herit. (Family-*Flacourtiaceae*), commonly known as 'Baichi' or 'Katai'. It is an indigenous medicinal plant widely distributed in Bangladesh and India.[119] This plant has been reported as an answer for the treatment of a variety of diseases and functional disorder. Fruits are used as appetizing, diuretic, digestive, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera.[119,120]

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Habitat and morphology description of *Flacourtia ramontchi*:

The leaves of the Indian plant are deciduous. Gamble says they fall in January-February, and the new foliage appears in April and May. Flowers from March-November. The trees growing in the forests of all over Bangladesh. The bark whitish-grey, says Kanjilal. Trimen says it is a small tree, with long simple spinous twigs on the young branches and often large, compound, branched spines on the trunk. Bark rather smooth, grey; young shoots pubescent. Leaves 2-3 in., broadly ovate, acuminate, obtuse, acute at base, more or less crenate-serrate, glabrous or pubescent on the veins beneath, thin. Petioles ¼ in., often pubescent. Flowers small, in little few-flowered axillary raceme clusters ; male flower sepals reflexed, ciliate ; female flower sepals very small, ciliate ; disk annular. Ovary globular; stigmas 5-6, nearly sessile, recurved. Berry globular, ½ in., diam., pulpy, smooth, marked with scars of fallen stigmas. Fruit red or brown, dark inky, when ripe. Seeds 4-6, strongly lobulated.

Distribution:

Flacourtia ramontchi is a common in tropical dry deciduous and thorn forests, though more abundant in the former. It also occurs in seasonally dry forest, woodland, bushland, thickets, wooded grassland, and often in riparian vegetation. The species is drought resistant though somewhat frost tender. The tree is usually leafless just before flowering. In

Bangladesh, the flowers appear from December to April together with the new leaves, which are a very beautiful fresh green colour. Fruits ripen from March to July. They are eaten by birds, thus the seeds are widely dispersed, accounting for the very wide distribution of the species.

Botanical information of *Flacourtia ramontchi*:[119]

Scientific classification

Kingdom:	Planeta
Division:	Angiosperms
Class:	Dicotyledons
Order:	Violales
Family:	*Flacourtiaceae*
Genus:	*Flacourtia Comm. ex L'Hér*
Species:	

Vernacular Name:[121]

Sanskrit	Vikankata, Gopakanta
Bengali	Bincha, Bainchi, Bewich
Hindi	Bilangra
Malayalam	Vavankataku , Vikamkath,
Marathi	Kaker
Gujarati	Kankata
Oriya	Kantheikoli, Vaincha, Uincha
Kannada	Llumanika, Dodda Gejjalakai
Tamil	Sottaikala, Kat Ukala

Medicinal importance and uses of *Flacourtia ramontchi*:

According to Sanskrit writers, the fruits are sweet, appetising and digestive. They are given in jaundice and enlarged spleen (U. C. Dutt) fter child-birth among natives in the Deccan, the seedsare ground to powder with turmeric, and rubbed all over the body to prevent rheumatic pains from exposure to damp winds (Dymock.). The gum is given along with other ingredients for cholera. The bark is applied to the body along with that of Albizzia, at intervals of a day or so during intermittent fever, in Chutia Nagpur (Revd. A. Campbell). The Species of Albizzia is not mentioned (K. R. K.). The phytochemical tests of *Flacourtia ramontchi* showed that the plant contain alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. The antioxidant test results that *Flacourtia* leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.[122] The pharmacological studies conformed the traditional uses of the Flacourtia indica as antioxidant, antimicrobial, anti asthamatic, anti diabetic, anti bacterial, anti inflamatory, anti malarial, hepatoprotective agent. Most of theraputic effects explained due to the presence of glycosides, tannins, Sugar, flacourtin, β-sitosterol, β-sitosterol-β-D-glucopyranoside, ramontoside, butyrolactone lignan disaccharide, coumarin such as scoparone and aesculetin etc.[123]

***Nyctanthes arbor-tristis* L.**

Nyctanthes arbor-tristis L. belongs to the family *Oleaceae* of the order Jasmlnaceae. Its synonyms are Sephalika, Parijatham in Sanskrit, Harsingar in Hindi and Coral / Night Jasmine in English. It is having brilliant, highly fragrant flowers which are white and yellow, and do not expand till evening and which fall off about sunrise. Thus during the day the plant lose all its brightness, and hence is called ‘the sad tree’. Nyctanthes means ‘Night-flowering’.[124] The indigenous people of Bangladesh widely use the whole plant for treatment of cancer, root for fever, sciatica, anorexia;

bark as expectorant, leaf for control fever, diabetes and as cholagogue, diaphoretic and anthelmintic. Various extracts of the plant is used to treat arthritis,[125] malaria, intestinal worms, tonic, laxative, antitrypanosomal, anti-inflammatory and antioxidant activity.[126,127,128]

Habitat and morphology description of *Nyctanthes arbor-tristis* L.:

Nyctanthes arbor-tristis Linn is a large shrub growing to 10 m tall, with flaky grey bark[129], stiff whitish hair, young branches[130] and rough leaves[131]. The flowers are fragrant, with a five- to eight-lobed white corolla with an orangered centre; they are produced in clusters of two to seven together, with individual flowers opening at dusk and finishing at dawn[129]. Calyx is 6-8 mm long, narrowly campanulate, hairy outside, glaborous inside truncate or obscurely toothed or lobed, ciliated. Corolla glaborous and is more than 13 mm long; tube is 6-8 mm long, orange coloured, about equalling the limbs; lobes are white and unequally obcordate and cuneate[130]. The leaves are opposite, simple, 6–12 cm long and 2–6.5 cm broad, with an entire margin. The fruit is a flat brown heart-shaped to round capsule 2 cm diameter, with two sections each containing a single seed[129]. These are long and broad, obcordate or nearly orbicular, compressed, 2-celled. Seeds are exalbuminous, testa are thick, outer layer of large transparent cells is heavily vascularised.[130]

.

Distribution:

Nyctanthes arbor-tristis Linn. is native to India, distributed widely in sub-Himalayan regions and southward to Godavari[131,132]. It is also distributed in Bangladesh, Indo-Pak subcontinent and South-East Asia[133], tropical and sub-tropical South East Asia[134]. It grows in Indo-Malayan region and distributed across Terai tracts as well as Burma and Ceylon[131]. It tolerates moderate shade and is often found as undergrowth in dry deciduous forests[135]

Botanical information of *Nyctanthes arbor-tristis* Linn:[128]

Scientific classification

Kingdom:	Planeta
Division:	Angiosperms
Class:	Eudicots
Order:	Lamiales
Family:	*Oleaceae*
Genus:	*Nyctanthes*
Species:	*N. arbor-tristis*

Vernacular Name:

Sanskrit	Parijatha
Bengali	Shephalika, Siuli
Hindi	Harashringara, Harsingar
Malayalam	Parijatakam
Marathi	Parijathak
Gujarati	Jayaparvati
Oriya	Gangasiuli
Kannada	Parijatha
Tamil	Parijata, Paghala

Medicinal importance and uses of *Nyctanthes arbor-tristis* Linn:

The flowers are used as stomachic, carminative, astringent to bowel, antibilious, expectorant, hair tonic and in the treatment of piles and various skin diseases[133] and in the treatment of ophthalmic purposes[130]. The bright orange corolla tubes of the flowers contain a coloring substance nyctanthin, which is identical with ά-Crocetin ($C_{20}H_{24}O_4$) from Saffron. The corolla tubes were formerly used for dyeing silk, sometimes together with Safflower or turmeric[135]**.** Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant[136]. The bark is used for the treatment of snakebite and bronchitis[133,137]. The stem bark pounded with *Zingiber officinale* and *Piper longumis* boiled in water and the resultant liquid is taken for two days for the treatment of malaria. The resulting paste on mixing with Arjuna bark is rubbed on the body to treat internal injury and for joint broken bones.[130] The leaves of *Nyctanthes arbor-tristis* L. are used extensively in Ayurvedic medicine for the treament of various diseases such as sciatica, chronic fever, rheumatism, and internal worm infections, and as a laxative, diaphoretic and diuretic[138]. Leaves are used in cough. Leaf juice is mixed in honey and given thrice daily for the treatment of cough. Paste of leaves is given with honey for the treatment of fever, high blood pressure and diabetes.[139] Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Leaves are also used in the enlargement of spleen.[136,140] The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors.[141] The extracted juice of leaves acts as a cholagogue, laxative and mild bitter tonic. It is given with little sugar to children as a remedy for intestinal ailments. In several cases, it has been found to act efficaciously for malaria fever.[135] The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative.[142]

Introduction and Objectives of the present work:

Medicinal plants are alleged to possess therapeutic effects in various diseases. From medicinal point of view, the great importance of active principles from bitter medicinal plants are specially notable. The important therapeutic agents like ribosome-inactivating proteins, carotenoids, carbohydrate binding lectin, antihyperglycemic agents, seed globulins, guanylate cyclase, antilipolytic and lipogenic agents, cytotoxic agents, antioxidants, trypsin inhibitor, ribonuclease, etc. are found in flowers, seeds, plants, leaves and roots of bitter plants. Seven bitter medicinal plants of Bangaldesh, viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L. have been selected for present investigation owing to their recognised medicinal importance. These plants are used in the treatment of bronchitis, stomachic, carminative, anthelmintic, fever, asthma, inflammation, febrifuge, gonorrhoea, antitumor, liver complaints, vomiting, leprosy, jaudice, dyspepsia, piles, antidiabelic, anti-cancer, leucoderma, lumours, gonorrhoea, rheumatism, diarrhoea, and many other diseases. Our present investigation deals with the extraction of the above medicinal plants with ethanol and microbiological, cytotoxic, haematological and histopathological investigations of the extracts of the above plants. The conceptual framework is shown in Figure- I.

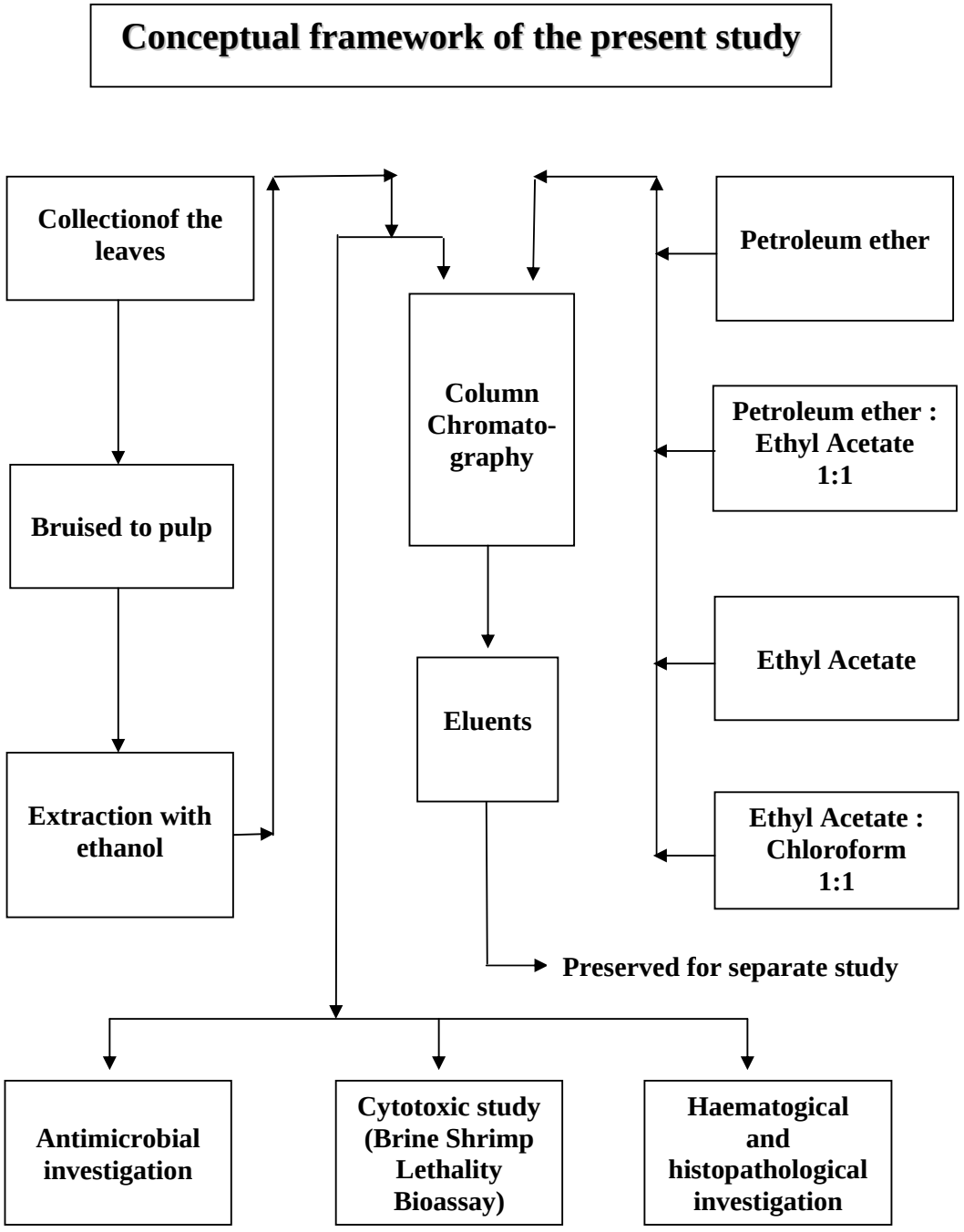


Fig-I: Conceptual framework of present study.

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Chapter II

Antimicrobial Investigations of Ethanol Extracts of the Plants

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

Chapter II

ANTIMICROBIAL INVESTIGATIONS OF ETHANOL EXTRACTS OF THE PLANTS:

IIA. Introduction:[142(A),143-145]

To a certain antibacterial spectrum of an agent against various types of pathogenic microorganisms, antibacterial screening is necessary. The susceptibility of microorganisms to the antimicrobial agents may be determined in vitro by a number of techniques among which the Disc diffusion method,[145] using different concentration of the agents absorbed on sterile filter paper disc, is widely acceptable for the preliminary investigation of materials which are suspected to possess antimicrobial properties.

Antimicrobial activity of the ethanol extracts were evaluated by 'Disc diffusion method.[145] Disc diffusion method is essentially a qualitative or semi quantitative test, which indicates the sensitivity or resistance of microorganism to the test material. However, no distinction between bacteriostatic and bactericidal activity can be made by this method.[146]

The microbiological investigation was done to determine the susceptibility of some pathogenic bacteria to ethanol extracts of the seven medicinal plants, viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L.. The importance of this medicinal plants in human civilization is very considerable. Human being and other living organisms are to depend, to a very great extent, on this type of medicinal plants.

IIB Principle[147] :

Dried filter paper discs impregnated separately with the test materials were placed gently on nutrient agar plates inoculated with the test organism. The dried discs absorbed water media and the test materials diffused. The special arrangement of the discs were such that they were not closer than 15 mm to the edge of the plate and far enough from each other to prevent overlapping of the zones of inhibition. These plates were then kept in a refrigerator at low temperature (4°C) for 24 hours to allow maximum diffusion. During this period dried discs absorb water from the media. The diffusion occurs according to the law that controls the diffusion of molecules through an agar gel. As a result there is a gradual change of test materials concentration in the media surrounding each disc. The plates were then incubated at 37.5°C for 24 hours to allow growth of the organisms. If there is any antimicrobial activity of test materials it will inhibit the growth of microorganisms and a clear zone of inhibition will be visualized surrounding the discs. The antimicrobial activity of the test materials is determined by measuring the diameter of zone of inhibition. The larger zone of inhibition is observed for more susceptible organism.

IIC. Materials and methods:

IIC.1 Collection of plants materials:

Leaves of seven medicinal plants viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L. under present investigation were collected from the different parts of Rajshahi district, Bangladesh.

IIC.2 Extraction of the powdered/pasty plant materials with ethanol:

The leaves of the above medicinal plants were washed separately with tap-water to remove adhering dirt. They were cut into small pieces with a knife. The chopped pieces were dried in the sun for four days. The sun dried materials were then dried in an electric oven at 40^{o}c for about 72 hours when the leaves became almost dry. These were then removed from the oven and the dried leaves were pulverized into powder and paste with the help of a grinding machine. The whole operations for the leaves of seven medicinal plants were done separately. The powdered and pasty leaves were stored in seven separate air tight containers and kept in a cold, dark place for investigation.

The powdered and pasty leaves were taken in seven separate quick-fit glass stoppered bottles. The contents of the bottles were shaken continuously with ethanol in an electric shaker for about nine hours. The bottles were allowed to stand for several days. The contents of the bottles were filtered successively in a tincture press and the filtrates were separately collected in seven glass containers. The ethanol extracts of seven medicinal plants were concentrated under reduced pressure when brownish-yellow semi-solid masses were obtained. Each of the concentrated extracts was preserved in a refrigerator for investigations. In every extraction, 250 g of powered or pasty leaves of the plants were taken in 500 ml ethanol.

A small portion of each of the concentrated extracts was separately resolved in column chromatography by eluating the chromatographic column with solvent systems (a) Petroleum ether, (b) Petroleum ether : ethyl acetate (1:1), (c) ethyl acetate, (d) ethyl acetate : chloroform (1:1). The eluants were concentrated to dryness. These eluants were separately preserved for further investigations which will form a separate study.

IIC.3 Apparatus, Chemicals and Reagents:

1. Ethanol extracts of seven medicinal plants.
2. Kanamycin (30µg/disc or 30µg/10µl) as standard antibiotic disc.
3. Blank sterilized filter paper discs (diameter 5mm).
4. Test tube.
5. Nutrient agar media.
6. Petridishes.
7. Beaker.
8. Sterile cotton.
9. Inoculating loop.
10. Sterile forceps.
11. Micro-pipette (10 µl-100 µl).
12. Bunsen burner.
13. Spreader.
14. Autoclave.
15. Incubator.
16. pH meter.
17. Distilled water.
18. Punch machine.
19. Ethanol (E. Merck, Germany).
20. Laminar air flow unit (Biocraft and Scientific Industries, India).
21. Refrigerator.
22. Solvents (Rectified spirit, Absolute ethanol, Methanol, etc).
23. Vials.

IIC.4 Test Organisms:

Four Gram-positive bacteria (*Bacillus megaterium*, *Staphylococcus aureus*, *Bacillus subtilis*, *Sarcina lutea*) and five Gram-negative bacteria (*Shigella shiga*, *Salmonila typhi-A*, *Escherichia coli*, *Shigella sonnei*, *Shigella dysenteiae*) were collected from the department of Pharmacy and the department of Biochemistry and Molecular Biology, University of Rajshahi.

List of the organisms (bacteria) used for determining the antibacterial activities of the extracts is given below.

List of Gram-positive bacteria

1. *Bacillus megaterium*.
2. *Staphylococcus aureus.*
3. *Bacillus subtilis*.
4. *Sarcina lutea.*

List of Gram-positive bacteria

1. *Shigella shiga*.
2. *Salmonila typhi-A*.
3. *Escherichia coli*.
4. *Shigella sonnei.*
5. *Shigella dysenteiae.*

IIC.5 Composition of the nutrient agar medium (for 1000 ml) :

For demonstrating the antibacterial activity and subculture of the test organism, the nutrient agar media having the following composition was used.

Ingredients	Amount
Beef extract	1.5 g
Peptone	5.0 gm
Sodium chloride	5.0 gm
Bacto yeast extract	2.0 gm
Bacto agar	15.0 gm
Distilled water q.s to	1000 ml

pH: 7.0±0.2 at 25^{o}C

IIC.6 Preparation of nutrient agar medium :

To prepare the required volume of the medium, the amount of each of the constituents was calculated from the composition that given for 1000ml. Peptone, sodium chloride, bacto yeast extract and beef extract of the calculated amount were taken in a conical flask after weighing separately. Demineralized water (volume was less than the required final volume) was added and the contents were heated in a water bath to make a clear solution. The pH was adjusted at 7.0 ± 0.2 using NaOH or HCI. The required amount of bacto agar was added to the solution and the demineralized water was added to make the final volume. Again the total volume was heated in a water bath to obtain a clear solution. The conical flask was plugged with cotton and then sterilized in an autoclave at a pressure of 15 lbs/inch^2 for 20 minutes at 121°C.

IIC.7 Preparation of inoculum :

The test organisms were transferred to the agar slants from the supplied pure cultures with the help of an inoculation loop in aseptic condition. For the growth of the test organisms the inoculated slants were incubated at 37.5°C for 24 hours. These fresh cultures were used for sensitivity test within 2 to 3 days.

IIC.8 Inoculation of nutrient agar plates:

The nutrient medium was poured into the petridishes on a level horizontal surface to give uniform depth of approximately 4 mm after the medium had been allowed to solidify. A suspension of fresh culture of the test organisms in the slants were transferred into the plates of different sterile petridishes containing nutrient agar medium so that it provided a uniform distribution of the of organism in the agar medium. An aseptic technique was followed. Petridishes were gently rotated first clock wise and then anti-clock wise so that a homogeneous distribution of the test organism was obtained. Just before use, the droplets of moisture on the surface of the petridishes were removed carefully. The petridishes, forceps, etc., were previously sterilized.

IIC.9 Preparation of discs :

Two types of discs were prepared for antibacterial and antifungal screening. These are sample discs and standard discs.

Sample discs :

Ethanol extracts of the leaves of seven medicinal plants were dissolved separately in sufficient amount of ethanol so that each 10µl of solutions contained 300 µg of the test materials. Filter paper discs were taken in cotton plugged test tube and was sterilized in autoclave at 121°C and at 15 lbs/inch^2 pressure for twenty minutes. Each of the filter paper discs was held up with a sterile forceps and then sterilized filter paper disc (5 mm diameter) were taken in a blank petridish. 10µl solution of the extract was applied on it with a micropipette and left for

sufficient time for complete evaporation of the solvent. Finally they were incubated at 37.5°C for 24 hours. Thus seven sample discs were prepared for seven separate plant extracts each containing 300 µg of the extracts.

Simultaneously, another 2 sets of similar experiments were separately performed for each of 400 µg and 500 µg ethanol extracts of the plants in 10 µl solution in identical conditions. Thus the sample discs consisting of 21 members for whole experiment contained the crude ethanol extracts of the leaves of seven medicinal plants having concentrations of 300 µg/10 µl, 400 µg /10 µl and 500 µg/10 µl for each of the plants extracts.

Standard disc :

These were used to compare the antibacterial and antifungal activities of the test materials. Standard antibiotic discs of Kanamycin (30 µg/disc) for antibacterial and antifungal activity were used for comparison. Filter paper along with residual solvent was used as negative control.

IIC.10 Placement of the discs, diffusion and incubation :

The sample discs and the standard antibiotic discs were placed gently on the solidified agar plates of different petridishes containing inoculated test organism with the help of sterile needle to ensure complete contact with the surface of the medium.

The plates were inverted and kept at 4^{o}C in a refrigerator for 24 hours to ensure the diffusion of the test materials and antibiotics from the impregnated disc to its surroundings. The petridishes, forceps, etc.were previously sterilized. Finally they were incubated at 37.5°C for 24 hours.

IIC.11 Measurement of the zones of inhibition :

After incubation for 24 hours, the antimicrobial activities of the crude ethanol extracts were estimated by measuring the diameter (mm) of the zone of inhibition of the organism. In each case, this was compared to that of standard sample. Filter paper along with residual solvent was used as negative control. The results were summarized in Tables: I-VIII.

II.D Results and discussion:

The antibacterial activity of the crude ethanol extracts obtained from the leaves of seven medicinal plants (viz, *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L.) were tested against nine bacteria at concentration of 300 μg/disc, 400 μg/disc and 500 μg/disc. Standard antibiotic disc kanamycin (30μg/disc) was used for comparison. The diameters of the zones of inhibition derived by ethanol extracts of the leaves of the plants were measured in mm with a transparent scale and the results were presented in Tables: I - VIII.

The diameters of the zones of inhibition derived by ethanol extract of the leaves of *Andrographis Paniculata* N. at 300 μg/disc concentration against *Bacillus megaterium*, *Staphylociccus aureus*, *Bacillus subtilis*, *Salmonella typhi, Escherichia coli, Shigella dysenteriae*, were found from the tables to be 11, 10, 9, 11, 10 and 12 respectively. At 400 μg/disc doses, the zones of inhibition produced by the extract against those bacteria were 12, 11, 10, 12, 12 and 14 mm respectively. At 500 μg/disc doses, the produced zones of inhibition by the extract against the same bacteria were recorded as 13, 12, 12, 16, 14 and 18 mm respectively. The extract showed maximum activity against *Shigella dysenteriae*. Moderate activities of the extract were observed against *Salmonella typhi, Escherichia coli, Bacillus megaterium*, *Staphylociccus aureus* and *Bacillus subtilis*. No activity of the extract on *Sarcina lutea, Shigella shiga* and *Shigella sonnei* was experienced.

The zones of inhibition produced by ethanol extract of the leaves of *Vinca rosea* L. at 300 μg/disc concentration against *Staphylociccus aureus*, *Bacillus subtilis*, *Sarcina lutea, Salmonella typhi, Escherichia coli* and *Shigella dysenteriae*, were 15, 16, 13, 14, 13 and 11 respectively. At 400 μg/disc concentration, the zones of inhibition produced by the extract against the same bacteria were found to be 17, 18, 16, 16, 15 and 13 mm respectively. At 500 μg/disc concentration, the produced zones of inhibition by the extract against these bacteria were tabulated as 19, 19, 17, 18, 19 and 16 mm respectively. The extract of *Vinca rosea* L. was active against *Staphylociccus aureus*, *Bacillus subtilis*, *Sarcina lutea, Salmonella typhi, Escherichia coli* and *Shigella dysenteriae*. The extract was found to posses the maximum activities on *Staphylociccus aureus*, *Bacillus subtilis* and *Escherichia coli*. The activity of the extract against *Bacillus megaterium, Shigella shiga* and *Shigella sonnei* was not found.

The zones of inhibition derived by ethanol extract of the leaves of *Adhatoda vasica* N. at 300 μg/disc concentration against *Bacillus megaterium*, *Staphylociccus aureus*, *Bacillus subtilis*, *Escherichia coli, Shigella sonnei* and *Shigella dysenteriae*, were observed to be 13, 11, 12, 12, 13 and 15mm respectively. At 400 μg/disc concentration, the zones of inhibition produced by the extract against these bacteria were 14 12, 13, 15, 14 and 16 mm respectively. At 500 μg/disc dose, the produced zones of inhibition by the extract against the same bacteria were noted as 16, 14, 14, 17, 16 and 18 mm respectively. The extract showed the maximum activities against *Shigella dysenteriae*. It had moderate effects on *Escherichia coli, Shigella sonnei, Bacillus megaterium*, *Staphylociccus aureus* and *Bacillus subtilis*. The extract had no or little activity against *Sarcina lutea*, *Shigella shiga* And *Salmonella typhi*.

The zones of inhibition obtained by ethanol extract of the leaves of *Vitex vegundo* L. at 300 μg/disc concentration against *Bacillus megaterium*, *Staphylociccus aureus*, *Bacillus subtilis*, *Salmonella typhi, Escherichia coli* and *Shigella dysenteriae*, were recorded as 13, 15, 15, 13, 12 and 11mm

respectively. At 400 μg/disc concentration, the produced zone of inhibition by the extract against the same bacteria were tabulated as 15 17, 16, 14, 14 and 13mm respectively. At 500 μg/disc dose, the observed zones of inhibition by the extract against these bacteria were 16, 18, 18, 17, 18 and 14mm respectively. The extract of *Vitex vegundo* L. showed the maximum activities on *Staphylociccus aureus*, *Bacillus subtilis* and *Escherichia coli*. The extract had a little or no activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*.

The zones of inhibition produced by ethanol extract of the leaves of *Aloe indica* W. at 300 μg/disc concentration against *Bacillus megaterium*, *Staphylociccus aureus*, *Bacillus subtilis*, *Salmonella typhi, Escherichia coli* and *Shigella dysenteriae*, were found to be 12, 9, 10, 12, 13 and 14mm respectively. At 400 μg/disc concentration, the zones of inhibition produced by the extract against the same bacteria were recorded as 12, 12, 13, 14, 14 and 16mm respectively. At 500 μg/disc dose, the observed zones of inhibition by the extract against these bacteria were 15, 13, 14, 15, 16 and 19mm respectively. The extract exhibited the maximum activities against *Shigella dysenteriae*. It had moderate activities against *Escherichia coli, Bacillus megaterium*, *Salmonella typhi, Bacillus subtilis* and *Staphylociccus aureus*. The extract showed no activity on *Sarcina lutea*, *Shigella shiga* and *Shigella sonnei*.

The zones of inhibition obtained by ethanol extract of the leaves of *Flacortia ramontchi* at 300 μg/disc concentration against *Bacillus megaterium*, *Staphylociccus aureus*, *Bacillus subtilis*, *Escherichia coli, Shigella sonnei* and *Shigella dysenteriae*, were recorded as 12, 13, 13, 14, 14 and 12mm respectively. At 400 μg/disc concentration, the derived zones of inhibition by the extract against the same bacteria were 13, 14, 15, 15, 16 and 14mm respectively. At 500 μg/disc dose, the observed zones of inhibition by the extract against these bacteria were recorded as 13, 15, 17, 18, 19 and 15mm respectively. The extract of *Flacortia ramontchi* showed maximum activity against *Shigella sonnei*. The moderate activities of the

extract were observed against *Escherichia coli, Bacillus subtilis*, *Staphylociccus aureus*, *Shigella dysenteriae* and *Bacillus megaterium*. The activity of the extract against *Sarcina lutea*, *Shigella shiga* and *Salmonella typhi* was not found.

The zones of inhibition produced by ethanol extract of the leaves of *Nyctanthes arbortristis* L. at 300 μg/disc concentration against *Staphylociccus aureus*, *Bacillus subtilis*, *Salmonella typhi, Escherichia coli, Shigella sonnei* and *Shigella dysenteriae*, were 21, 13, 20, 17, 16 and 17mm respectively. At 400 μg/disc concentration, the inhibited zones produced by the extract against the same bacteria were tabulated as 23, 26, 24, 18, 20 and 19mm respectively. At 500 μg/disc dose, the observed zones of inhibition by the extract against these bacteria were 25, 28, 28, 24, 26 and 21mm respectively. The extract of *Nyctanthes arbortristis* L.was very active against *Bacillus subtilis* and *Salmonella typhi*. The extract had moderate activities on *Shigella sonnei, Staphylociccus aureus*, *Escherichia coli* and *Shigella dysenteriae*. The extract experienced no activity against *Bacillus megaterium*, *Sarcina lutea* and *Shigella shiga*.

II.E Conclusion:

From the foregoing evidences, we can conclude that the ethanol extracts of the plants under present investigation possess appreciable antibacterial activities.

Table-I :Antibacterial activity of the crude ethanol extract of *Andrographis paniculata* N.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract μg/10μl			Kanamycin 30 μg/10μl
	300	400	500	
Gram positive				
Bacillus megaterium	11	12	13	18
Staphylococcus aureus	10	11	12	23
Bacillus subtilis	09	10	12	17
Sarcina lutea	-	-	-	18
Gram negative				
Shigella shiga	-	-	-	19
Salmonila typhi-A	11	12	16	24
Escherichia coli	10	12	14	23
Shigella sonnei	-	-	-	16
Shigella dysenteiae	12	14	18	20

Table-II :Antibacterial activity of the crude ethanol extract of *Vinca rosea* L.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract μg/10μl			Kanamycin 30 μg/10μl
	300	400	500	
Gram positive				
Bacillus megaterium	-	-	-	23
Staphylococcus aureus	15	17	19	30
Bacillus subtilis	16	18	19	24
Sarcina lutea	13	16	17	20
Gram negative				
Shigella shiga	-	-	-	22
Salmonila typhi-A	14	16	18	25
Escherichia coli	13	15	19	23
Shigella sonnei	-	-	-	21
Shigella dysenteiae	11	13	16	23

Table-III :Antibacterial activity of the crude ethanol extract of *Adhatoda vasica* Nees.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract μg/10μl			Kanamycin 30 μg/10μl
	300	400	500	
Gram positive				
Bacillus megaterium	13	14	16	24
Staphylococcus aureus	11	12	14	31
Bacillus subtilis	12	13	14	27
Sarcina lutea	-	-	-	21
Gram negative				
Shigella shiga	-	-	-	22
Salmonila typhi-A	-	-	-	29
Escherichia coli	12	15	17	23
Shigella sonnei	13	14	16	26
Shigella dysenteiae	15	16	18	21

Table-IV :Antibacterial activity of the crude ethanol extract of *Vitex vegundo* Linn.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract μg/10μl			Kanamycin 30 μg/10μl
	300	400	500	
Gram positive				
Bacillus megaterium	13	15	16	20
Staphylococcus aureus	15	17	18	31
Bacillus subtilis	15	16	18	24
Sarcina lutea	-	-	-	21
Gram negative				
Shigella shiga	-	-	-	20
Salmonila typhi-A	13	14	17	26
Escherichia coli	12	14	18	23
Shigella sonnei	-	-	-	20
Shigella dysenteiae	11	13	14	19

Table-V :Antibacterial activity of the crude ethanol extract of *Aloe indica* W.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract μg/10μl			Kanamycin 30 μg/10μl
	300	400	500	
Gram positive				
Bacillus megaterium	12	12	15	19
Staphylococcus aureus	09	12	13	27
Bacillus subtilis	10	13	14	17
Sarcina lutea	-	-	-	20
Gram negative				
Shigella shiga	-	-	-	19
Salmonila typhi-A	12	14	15	30
Escherichia coli	13	14	16	23
Shigella sonnei	-	-	-	17
Shigella dysenteiae	14	16	19	19

Table-VI :Antibacterial activity of the crude ethanol extract of *Flacourtia ramontchi* L.

Name of bacteria	Diameter of the zone of inhibition in mm after 24 hours of incubation			
	Ethanol extract µg/10µl			Kanamycin 30 µg/10µl
	300	400	500	
Gram positive				
Bacillus megaterium	12	13	13	24
Staphylococcus aureus	13	14	15	28
Bacillus subtilis	13	15	17	30
Sarcina lutea	-	-	-	22
Gram negative				
Shigella shiga	-	-	-	21
Salmonila typhi-A	-	-	-	30
Escherichia coli	14	15	18	19
Shigella sonnei	14	16	19	23
Shigella dysenteiae	12	14	15	20

Table-VII: Antibacterial activity of the crude ethanol extract of *Nyctanthes arbortristis* Linn.

Name of bacteria	**Diameter of the zone of inhibition in mm after 24 hours of incubation**			
	Ethanol extract μg/10μl			**Kanamycin 30 μg/10μl**
	300	**400**	**500**	
Gram positive				
Bacillus megaterium	-	-	-	20
Staphylococcus aureus	21	23	25	27
Bacillus subtilis	23	26	28	31
Sarcina lutea	-	-	-	17
Gram negative				
Shigella shiga	-	-	-	19
Salmonila typhi-A	20	24	28	29
Escherichia coli	17	18	24	25
Shigella sonnei	16	20	26	29
Shigella dysenteiae	17	19	21	22

Table-VIII : Antimicrobial activities of ethanol extracts of the leaves of *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L.with respect to standard Kanamycin (30 µg/disc)

List of Gram-positive and Gram-negative bacteria		Diameter of zone of inhibition in mm after 24 hours of incubation															
		Andrographis paniculata N.				*Vinca rosea* L.				*Adhatoda vasica* N.				*Vitex vegundo* L.			
		Ethanol extracts µg/disc			Kana-mycin 30µg/ disc	Ethanol extracts µg/disc			Kana-mycin 30µg/ disc	Ethanol extracts µg/disc			Kana-mycin 30µg/ disc	Ethanol extracts µg/disc			Kana-mycin 30µg/ disc
		300	400	500		300	400	500		300	400	500		300	400	500	
Gram-positive bacteria	*Bacillus megaterium*	11	12	13	18	-	-	-	23	13	14	16	24	13	15	16	20
	Staphylococcus aureus	10	11	12	23	15	17	19	30	11	12	14	31	15	17	18	31
	Bacillus subtilis	09	10	12	17	16	18	19	24	12	13	14	27	15	16	18	24
	Sarcina lutea	-	-	-	18	13	16	17	20	-	-	-	21	-	-	-	21
Gram-negative bacteria	*Shigella shiga*	-	-	-	19	-	-	-	22	-	-	-	22	-	-	-	20
	Salmonila typhi-A	11	12	16	24	14	16	18	25	-	-	-	29	13	14	17	26
	Escherichia coli	10	12	14	23	13	15	19	23	12	15	17	23	12	14	18	23
	Shigella sonnei	-	-	-	16	-	-	-	21	13	14	16	26	-	-	-	20
	Shigella dysenteiae	12	14	18	20	11	13	16	23	15	16	18	21	11	13	14	19

Table-VIII (Continued) : Antimicrobial activities of ethanol extracts of the leaves of *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L. with respect to standard Kanamycin (30 µg/disc)

List of Gram-positive and Gram-negative bacteria		Diameter of zone of inhibition in mm after 24 hours of incubation											
		Aloe indica W.				*Flacortia ramontchi*				*Nyctanthes arbortristis* L.			
		Ethanol extracts µg/disc			Kana-mycin 30µg/ disc	Ethanol extracts µg/disc			Kana-mycin 30µg/ disc	Ethanol extracts µg/disc			Kana-mycin 30µg/ disc
		300	400	500		300	400	500		300	400	500	
Gram-positive bacteria	*Bacillus megaterium*	12	12	15	19	12	13	13	24	-	-	-	20
	Staphylococcus aureus	09	12	13	27	13	14	15	28	21	23	25	27
	Bacillus subtilis	10	13	14	17	13	15	17	30	23	26	28	31
	Sarcina lutea	-	-	-	20	-	-	-	22	-	-	-	17
Gram-negative bacteria	*Shigella shiga*	-	-	-	19	-	-	-	21	-	-	-	19
	Salmonila typhi-A	12	14	15	30	-	-	-	30	20	24	28	29
	Escherichia coli	13	14	16	23	14	15	18	19	17	18	24	25
	Shigella sonnei	-	-	-	17	14	16	19	23	16	20	26	29
	Shigella dysenteiae	14	16	19	19	12	14	15	20	17	19	21	22

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Chapter III

Determination of Minimum Inhibitory Concentrations of Ethanol Extracts

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

Chapter III

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATIONS OF ETHANOL EXTRACTS:

IIIA. Introduction:

Minimum inhibitory concentration (MIC) is the lowest concentration of an active principle at which the growth of microorganisms is completely inhibited. The MIC of a sample is determined by the following two methods.

(A) Serial tube dilution technique[148] and

(B) Paper disc plate technique or agar diffusion assay.[149]

IIIB. Materials and Methods:

The extracts of seven medicinal plants; apparatus, chemicals and reagent; test organisms; composition and preparation of nutrient agar medium, preparation of inoculum; inoculation of nutrient agar plates; preparation of sample discs and standard discs; placement of the discs, diffusion and incubation and measurement of the zones of inhibition were similar to those as described in the previous chapter (Chapter-II).

(A) Serial tube dilution technique [148]:

The minimum inhibitory concentration of the crude ethanol extract was determined against nine test organisms mentioned earlier. Nutrient broth medium and culture media were prepared earlier following the standard methods. Fresh culture of the pathogenic microorganisms, preparation of test samples, placement of the discs and incubation were achieved as described in the previous experiment. Kanamycin (30 μg/disc) was used as standard disc.

Crude ethanol extracts of the leaves of the above medicinal plants were transferred in seven separate vials containing 2% DMSO solution (2ml). This was mixed well to achieve sample solutions having concentration 1024 μg/ml. Nine sterilized test tubes containing 1 μg/ml, 2 μg/ml, 4μg/ml, 8μg/ml, 16μg/ml, 32μg/ml, 64μg/ml, 128μg/ml and 256μg/ml sample solutions were prepared for each of the 7 ethanol extracts by SDT[148]. Nine test tubes containing ethanol extracts of *Andrographis paniculata* N. having the above concentrations were considered for determining the MIC against *Bacillus megaterium*. Three test tubes containing media (C_M), media plus sample (C_{MS}) and media plus inoculums (C_{MI}) were also maintained for the experiments. Diluted inoculums (10μl) was added to each of the nine test tubes and mixed well. One ml of the sample was added to C_{MS} and mixed well. 10μl of inoculums of *Bacillus megaterium* was added to C_{MI} to observe the growth of the organisms in the media. C_M containing media was used the check the sterility of the solution. Twelve test tubes were incubated at 37.5^oC for 24 hours. After incubation, the test tubes were examined for visible growth of the organisms. The lowest concentration of the test material at which there was no visible growth was recorded as the MIC of the test material for the test organisms. The first sign of inhibition of test organisms by the ethanol extract of *Andrographis paniculata* N. was visualized in the test tubes and recorded. Similar 8 experiments were performed for the same extract against remaining 8 bacteria and MIC of the particular extract against above nine bacteria were recorded.

Similar experimental procedures were followed for the determination of MIC of remaining 6 extracts (viz, extracts of *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L.) against nine bacteria mentioned above. Thus the MIC of seven ethanol extracts of the leaves of above seven plants against nine bacterial strains were determined and the results were recorded in 63 tables. The results in brief were computed in Tables IX(A) and IX(B).

IIIC. Results and Discussion:

The minimum inhibitory concentration (MIC) of ethanol extracts of the leaves of the above medicinal plants against four gram-positive bacteria and five gram-negative bacteria were determined by serial tube dilution technique and the data were recorded in 63 separate tables. The results in brief were summarized in Tables: IX(A) and IX(B) to show the MIC of the extracts against the bacterial strains. It is evident from the table that all the extracts have notable antimicrobial activities against the test organisms. Appreciable MIC of the extracts has been enunciated in the table. The first sign of inhibition of organisms was seen in the test tube against the respective extracts having lower concentrations than those cited in the table had no inhibitory activities on the organisms.

IIID. Conclusion:

The MIC values of the extracts have remarkable significance about the therapeutic effects of the active principles associated with the leaves of the plants.

Table- IX(A) : Minimum inhibitory concentration (µg/disc) of the crude ethanol extracts of *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L.

List of Gram-positive and Gram-negative bacteria		Name of the plants and MIC (µg/ml) of crude ethanol extracts after 24 hours of incubation						
		Andrographis paniculata N.	*Vinca rosea* L.	*Adhatoda vasica* N.	*Vitex vegundo* L.	*Aloe indica* W.	*Flacortia ramontchi*	*Nyctanthes arbortristis* L.
		MIC	MIC	MIC	MIC	MIC	MIC	MIC
Gram-positive bacteria	*Bacillus megaterium*	32	-	32	64	32	128	-
	Staphylococcus aureus	128	64	128	32	128	64	64
	Bacillus subtilis	128	32	128	64	128	32	32
	Sarcina lutea	-	128	-	-	-	-	-
Gram-negative bacteria	*Shigella shiga*	-	-	-	-	-	-	-
	Salmonila typhi-A	64	32	-	32	64	-	32
	Escherichia coli	64	64	64	128	32	64	128
	Shigella sonnei	-	-	64	128	-	32	128
	Shigella dysenteiae	32	128	32	128	128	128	64

Table- IX(B) : Minimum inhibitory concentration (μg/disc) of the crude ethanol extracts of *Andrographis paniculata* N., *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi* and *Nyctanthes arbortristis* L.

List of Gram-positive and Gram-negative bacteria		Name of the plants and MIC (μg/ml) of crude ethanol extracts after 24 hours of incubation						
		Andrographis paniculata N.	*Vinca rosea* L	*Adhatoda vasica* N.	*Vitex vegundo* L.	*Aloe indica* W.	*Flacortia ramontchi*	*Nyctanthes arbortristis* L..
Gram-positive bacteria	*Bacillus megaterium*	+ + +	–	+++	++	+++	+	–
	Staphylococcus aureus	+	++	+	+++	+	++	++
	Bacillus subtilis	+	+++	+	++	+	+++	+++
	Sarcina lutea	–	+	–	–	–	–	–
Gram-negative bacteria	*Shigella shiga*	–	–	–	–	–	–	–
	Salmonila typhi-A	++	+++	–	+++	++	–	+++
	Escherichia coli	++	++	++	+	+++	++	+
	Shigella sonnei	–	–	+ +	+	–	+++	+
	Shigella dysenteiae	+++	+	+++	+	+	+	++

High antimicrobial activity ≡ + + +
Moderate antimicrobial activity ≡ + +
Low antimicrobial activity ≡ +
No antimicrobial activity ≡ –

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Chapter IV

Cytotoxic Effects of Ethanol Extracts of The Plants

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

Chapter IV

CYTOTOXIC EFFECTS OF ETHANOL EXTRACTS OF THE PLANTS

IV. Brine Shrimp Lethality : A Rapid General Bioassay For Cytotoxic Effect

IV.A Introduction :

Brine Shrimp Lethality bioassay[150] indicates cytotoxicity and a wide range of pharmacological activities of the compounds as well. This is a simple method which involves no aseptic technique. It utilizes a large number of organisms for statistical validation and a relatively small amount of sample. It does not require animal serum as it is needed for the other methods of cytotoxicities. Generally the median effective dose (ED_{50}) values for cytotoxicities are one tenth of median Lethal Concentration (LC_{50}) values in the Brine Shrimp bioassay.[151-155] This can be accompanied by testing in vitro lethality in a simple zoologic organism, viz, Brine Shrimp nauplii, Leach (Artemia Salina). In this method, bioactivity can be found not only for natural product extracts but also for pure compounds.

IV.B Materials and Methods:

IV.B.1 Materials

A. i) Artemia Salina Leach (Brine Shrimp eggs)

ii) Sea Salt

iii) Test sample (Ethanol extracts of the plants).

B. i) Small tank with perforated dividing dam to grow Shrimp and lamp to attract Shrimp.

ii) Pipettes (1ml, 5ml)

iii) Micropipettes (10 μl, 200 μl)

iv) Vials (5 ml)

IV.B.2 Experimental

IV.B.2.1 Preparation of sea water

Since the lethality test involves the culture of Brine Shrimp nauplii that is, the nauplii should be grown in the sea water. Sea water contains 3.8% of NaCl. Accordingly 3.8% Sodium chloride solution was made by dissolving Sodium chloride (38gm) in distilled water (1000 ml) and was filtered off. The pH of the brine water thus prepared was maintained between 8 and 9 using $NaHCO_3$

IV.B.2.2 Hatching of brine shrimp

A small tank with perforated dividing dam was filled in with required amount of brine water. Constant oxygen supply, sufficient aeration and constant temperature (37^oC) were accordingly maintained. Shrimp eggs (1.5g/l) were added to the one side of the divided tank. Two days were needed for the shrimps to hatch and mature as nauplii (Larvae). The hatched shrimp nauplii (Larvae) were attracted to the lamp on the other side of the divided tank through the perforations in the dam. These nauplii were taken for bioassay.

IV.B.2.3 Preparation of sample solution

The test samples under investigation were

i. Ethanol extracts of seven medicinal plants.

ii. Chloroform

10 mg of each of ethanol extracts was accurately weighed and dissolved in 1 ml DMSO (dimethyl sulfoxide) separately. Thus a concentration of 10 mg/ml was obtained which was used as stock solution A. Then a series of concentrations for each of the extracts were made from the stock solution.

i. 2.5 μg/ml

ii. 5 μg/ml

iii. 10 μg/ml

iv. 20 μg/ml

v. 40 μg/ml

vi. 80 μg/ml

vii. 120 μg/ml

From stock solution A, 10 μl was taken and diluted up to 5 ml. Since stock solution A had a concentration 10 mg /ml.

1 ml or 1000 μl contained 10 mg or 10000 μg

So, 1 μl contained = 10000/1000 μg

So, 10 μl contained = 10000×10/1000 μg

= 100 μg

As it was diluted up to 5 ml

5 ml contained = 100 μg

So, 1 ml contained = 100/5 μg

So, the concentration 20 μg/ml was obtained

Similarly 20 μl and 40 μl were taken respectively to get a concentration of about 40 μg/ml and 80 μg/ml.

As each 5 ml test solution should not contain more than 50 μl of DMSO it is necessary to dilute stock solution A with sea water to get lower concentrations, i.e., 2.5 μg/ml, 5 μg/ml and 10 μg/ml. The volumes of DMSO in these vials should not exceed 10μg/ml of the brine solution, because above this concentration toxicity due to DMSO may arise.

From stock solution A, 1 ml was diluted up to 8 ml with sea water, and this solution was marked as stock solution B. Thus, 10 μl, 20 μl and 40 μl from stock solution B diluted each up to 5 ml with sea water gave concentrations 2.5 μg/ml, 5 μg/ml and 10μg/ml, respectively.

With the help of a micropipette 10 µl, 20 µl, 40 µl, 80 µl and 120 µl of each of the samples was transferred from the stock solution in 5 different vials. Seawater (brine water) was added to each vial making the volume up to 5 ml. The final concentration of the samples in these vials becomes 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml and 120 µg/ml respectively. For each concentration, control experiment was performed.

For each concentration, 2 samples were prepared to get more accurate result and the average of these two were taken into account to determine the rate of mortality of nauplii.

IV.B.2.4 Preparation of the negative control group:

For each concentration, one vial containing the same volume of DMSO diluted upto 5 ml with sea water and 10 shrimps nauplii was used as negative control. It was used to verify the validity of the test. When the nauplii in the control showed a rapid mortality, then test is considered to be invalid as the nauplii might die due to reasons other than cytotoxicity of the compounds.

IV.B.2.5 Application of brine shrimp nauplii to the vials:

To each of the 5 sample vials contained different concentrations of ethanol extract of *Andrographis paniculata* N. and also to the control vials, 5 ml Brine Shrimp solution (3.8%) containing 20 alive napulii were added. The final concentrations of sea salt in 5 vials should be maintained 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml and 120 µg/ml respectively. If the counting of 20 napulii was not be possible accurately, then a variation in counting from 18-22 might be allowed. A magnifying glass was used for convenient counting of the nauplii.

IV.B.2.6 Counting of nauplii

After 24 hours, the vials were observed and the number of survived nauplii in each vial were counted and the results were noted. From this data, percentage of mortality of nauplii was calculated at each concentration for each sample. Similar experiments for remaining six ethanol extracts of *Vinca rosea* L., *Adhatoda vasica* N., *Vitex vegundo* L., *Aloe indica* W., *Flacortia ramontchi a*nd *Nyctanthes arbortristis* L. were performed in identical conditions and the results were summarized in Tables X-XVI. Corresponding graphs from the tables were prepared which had been computed in Figures 9-15.

IV.B.2.7 Analysis of data:

The concentration-mortality relationship of the ethanol extracts of the leaves may be expressed as a median Lethal Concentration value (LC_{50}). This indicates the concentration of active principles in each of the extracts which produces death in half of the test shrimp nauplii (Larvae) after a certain exposure period.

IV.C Results and discussion:

Brine Shrimp Lethality test is a positive correlation between Brine Shrimp toxicity and cytotoxicity. The crude ethanol extracts of seven medicinal plants under present investigation represented positive response to Brine Shrimp Lethality Bioassay. Lethal concentration (LC_{50}) of Brine Shrimp Lethality was determined to evaluate the cytotoxic effect of ethanol extracts. LC_{50} of Brine Shrimp Lethality was obtained from a plot of percentage of mortality versus concentration of the extracts on the graph. The results were represented in Tables X-XVI containing seven corresponding graphs (Figures 9-15). The results in the tables and graphs established a positive correlation between Brine Shrimp Lethality and

cytotoxicity. Brine Shrimp Lethality bioassay resembled that the ethanol extract the leaves of *Andrographis paniculata* N. is more cytotoxic (LC_{50}= 30 μg/ml) than those of other extracts. The very low LC_{50} indicated the high toxic effect of the extract of the leaves of the plant. Low cytotoxic effect of each of *Vitex vegundo* L. and *Nyctanthes arbortristis* L. (LC_{50}= 72 μg/ml) was observed. The other ethanol extracts exhibited moderate cytotoxic effects. Thus, we may conclude that the ethanol extracts of the leaves of seven medicinal plants under present investigation have cytotoxic effects.

IV.D Conclusion:

From the foregoing evidences, we can conclude that the ethanol extracts of the plants under present investigation possess appreciable antibacterial activities. The MIC values and cytotoxic effects of the extracts have remarkable significance about the therapeutic effects of the active principles associated with the leaves of the plants.

Table-X: Effect of ethanol extract obtained from the leaves of *Andrographis paniculata* N. against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	15	05	25	30
	20	20	12	08	40	
	40	20	10	12	60	
	80	20	02	18	90	
	120	20	00	20	100	

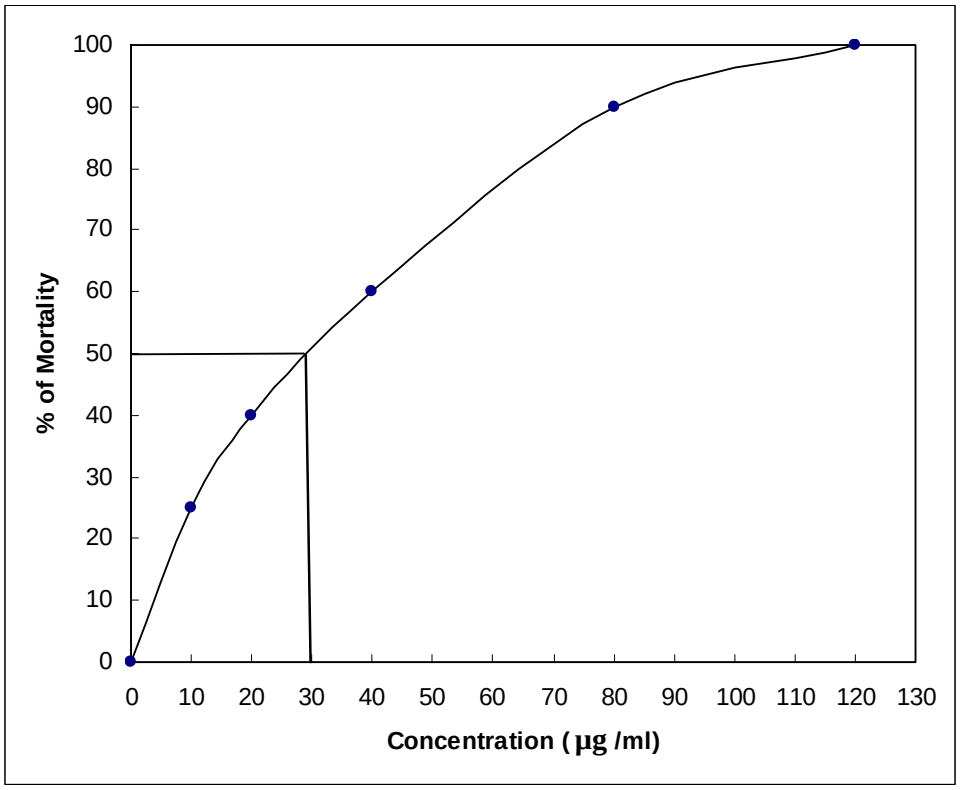


Fig-9: Determination of LC_{50} of ethanol extract of the leaves of *Andrographis paniculata* N. against Brine Shrimp nauplii.

Table-XI: Effect of ethanol extract obtained from the leaves of *Vinca rosea* L. against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	17	03	15	40
	20	20	15	05	25	
	40	20	10	10	50	
	80	20	03	17	85	
	120	20	01	19	95	

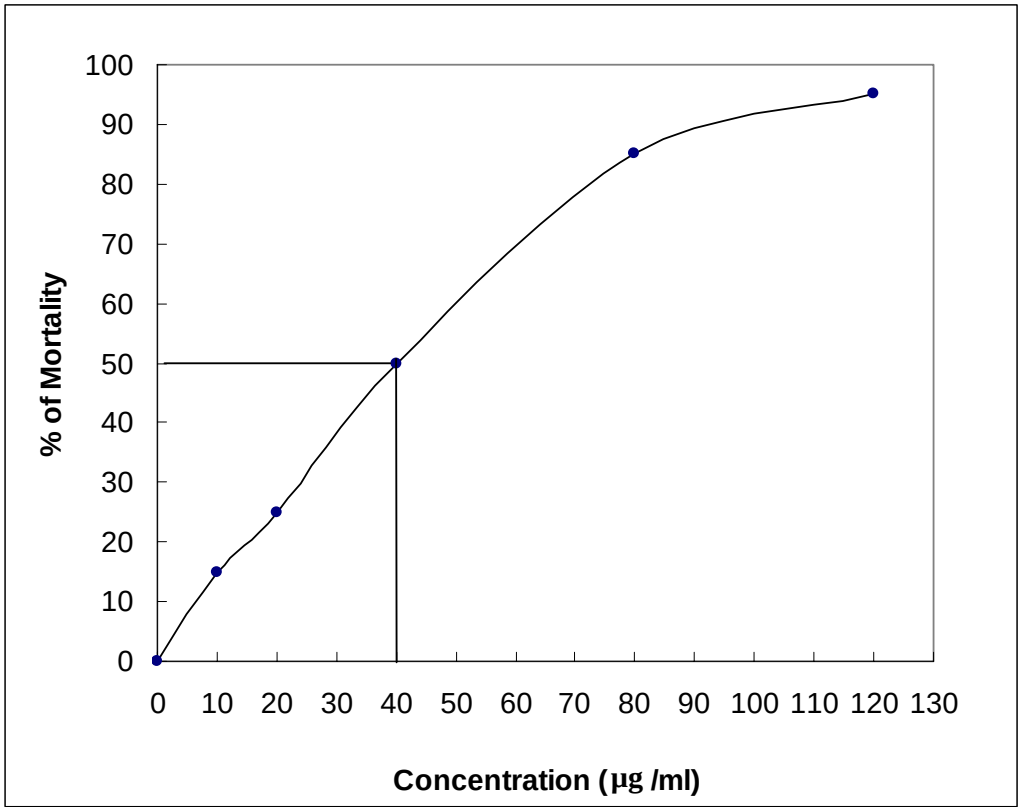


Fig-10: Determination of LC_{50} of ethanol extract of the leaves of *Vinca rosea* L. against Brine Shrimp nauplii.

Table-XII: Effect of ethanol extract obtained from the leaves of *Adhatoda vasica* N. against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	18	02	10	55
	20	20	16	04	20	
	40	20	13	07	40	
	80	20	07	13	65	
	120	20	04	16	80	

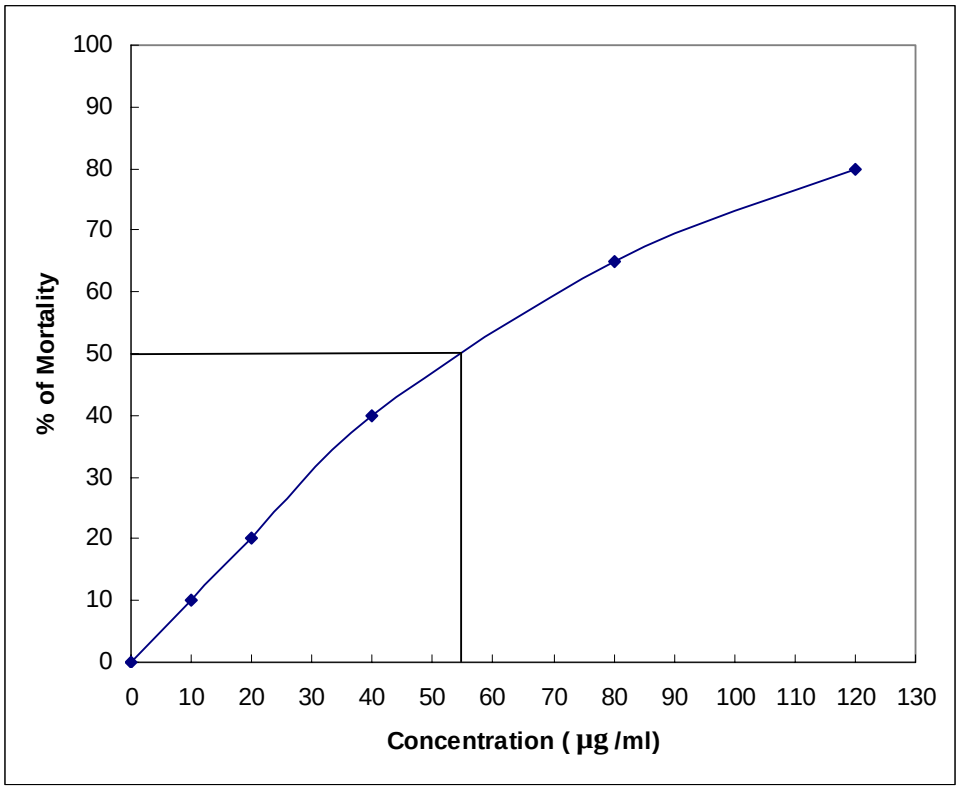


Fig-11: Determination of LC_{50} of ethanol extract of the leaves of *Adhatoda vasica* N. against Brine Shrimp nauplii.

Table-XIII: Effect of ethanol extract obtained from the leaves of *Vitex vegundo* L. against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	19	01	05	72
	20	20	17	03	15	
	40	20	14	06	30	
	80	20	09	11	55	
	120	20	05	15	75	

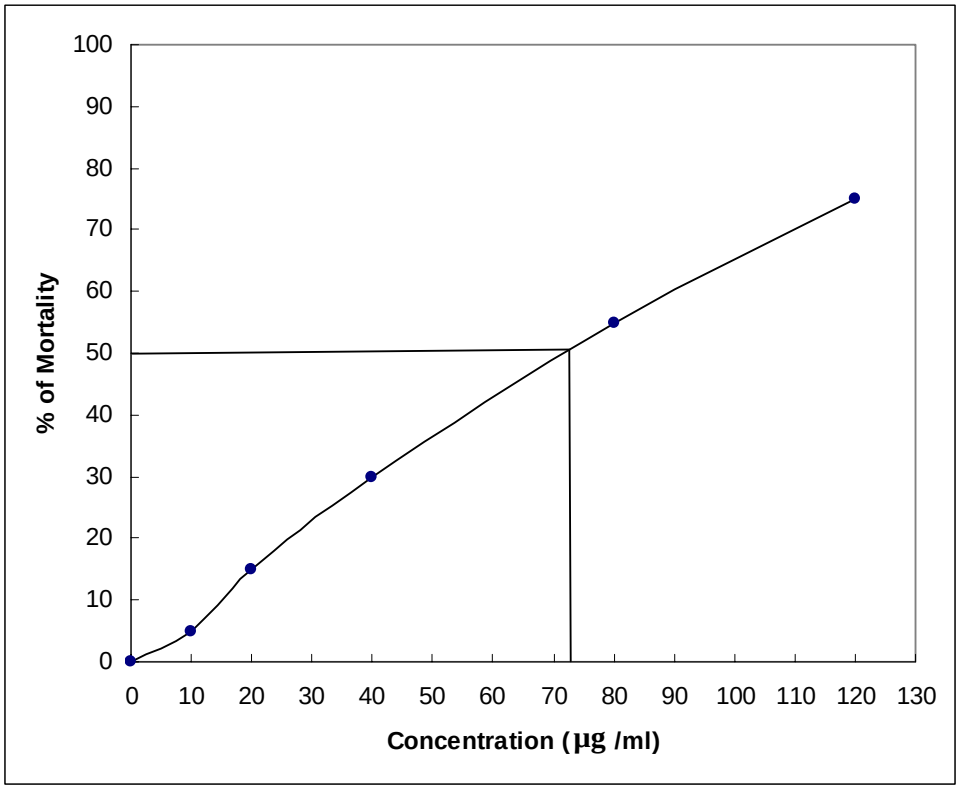


Fig-12: Determination of LC_{50} of ethanol extract of the leaves of *Vitex vegundo* L. against Brine Shrimp nauplii.

Table-XIV: Effect of ethanol extract obtained from the leaves of *Aloe indica* W. against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	18	12	10	58
	20	20	15	05	25	
	40	20	12	08	40	
	80	20	08	12	60	
	120	20	06	14	70	

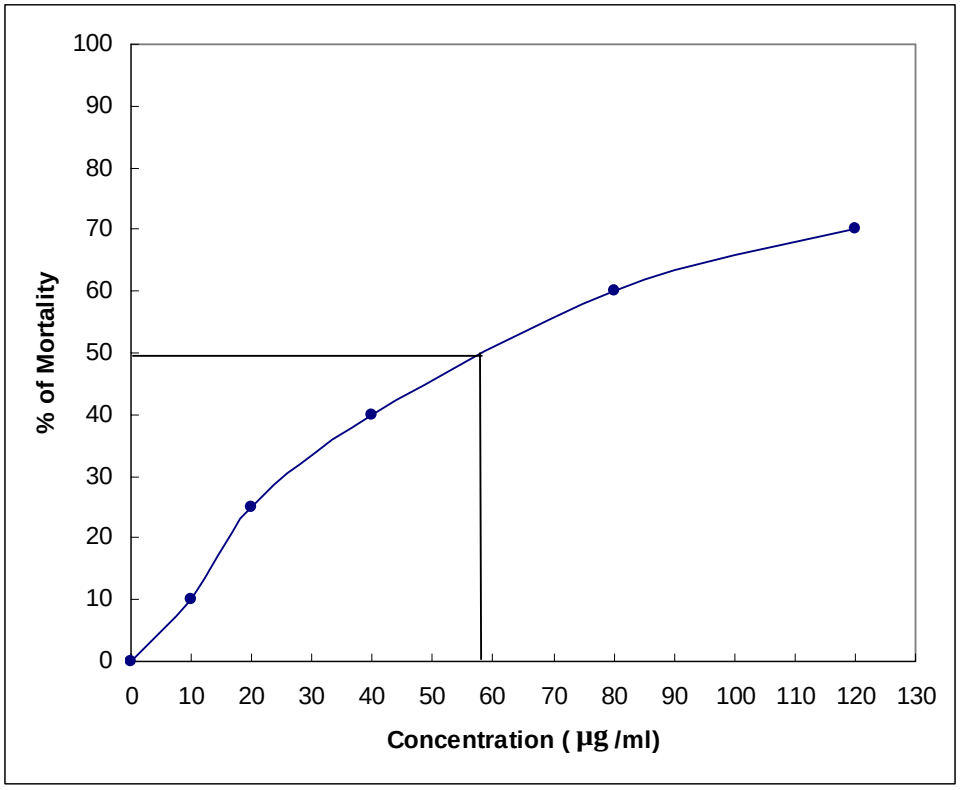


Fig-13: Determination of LC_{50} of ethanol extract of the leaves of *Aloe indica* W. against Brine Shrimp nauplii.

Table-XV: Effect of ethanol extract obtained from the leaves of *Flacortia ramontchi* against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	18	02	10	40
	20	20	14	06	30	
	40	20	10	10	50	
	80	20	07	13	65	
	120	20	04	16	80	

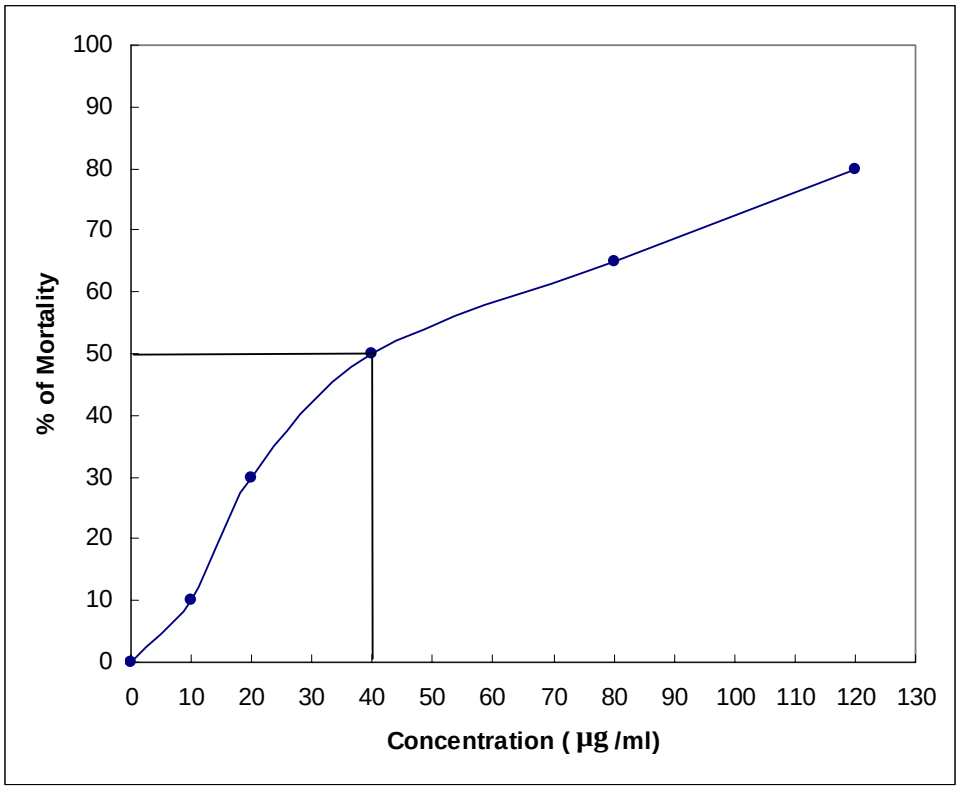


Fig-14: Determination of LC_{50} of ethanol extract of the leaves of *Flacortia ramontchi* against Brine Shrimp nauplii.

Table-XVI: Effect of ethanol extract obtained from the leaves of *Nyctanthes arbortristis* L.against brine shrimp nauplii.

Sample	Concentration µg/ml	No. of nauplii taken	No. of nauplii alive	No. of nauplii died	% of Mortality	LC_{50} µg/ml
Ethanol extracts	10	20	19	01	05	72
	20	20	17	03	15	
	40	20	14	06	30	
	80	20	09	11	55	
	120	20	03	17	85	

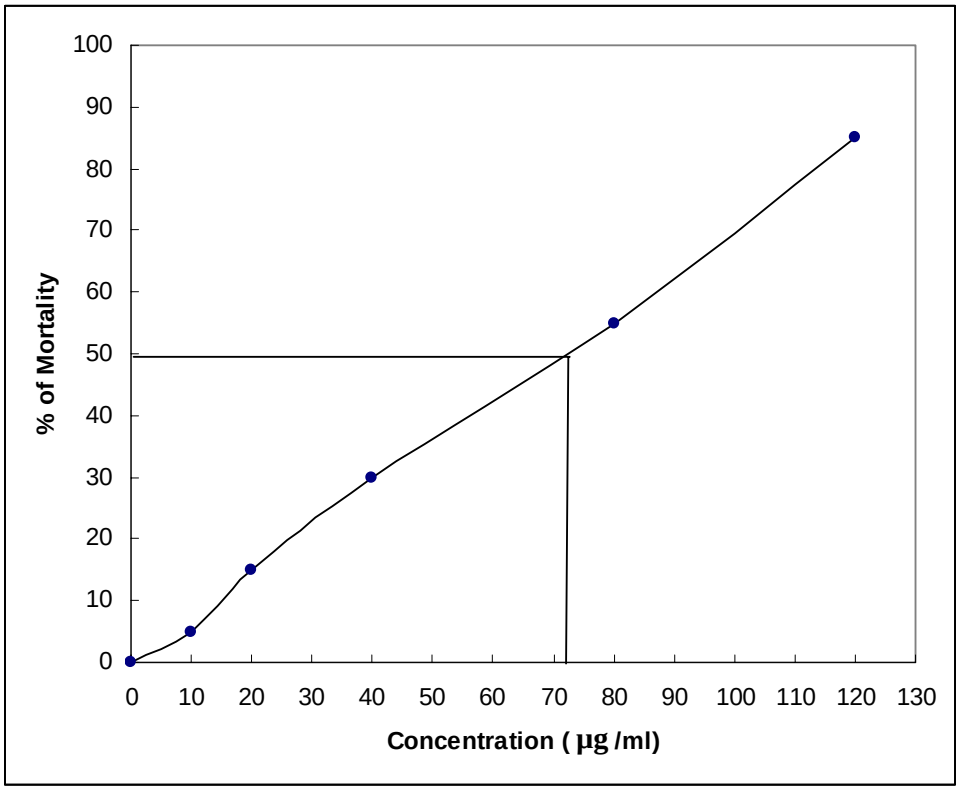


Fig-15: Determination of LC_{50} of ethanol extract of the leaves of *Nyctanthes arbortristis* L. against Brine Shrimp nauplii.

Andrographis paniculata **N.**

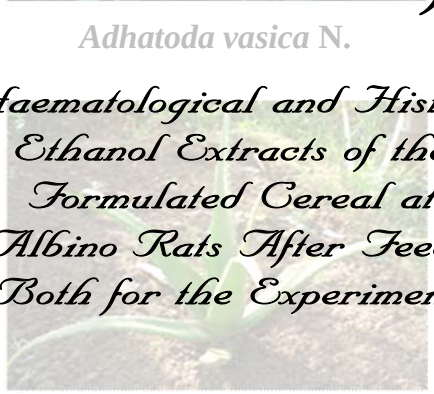
Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Chapter V

Haematological and Histopathological Investigations of Ethanol Extracts of the Plants Supplemented with Formulated Cereal at 5% Level on Young Male Albino Rats After Feeding for 28 Consecutive Days Both for the Experiment Group and Control Group.



Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

Chapter V

HAEMATOLOGICAL AND HISTOPATHOLOGICAL INVESTIGATIONS OF ETHANOL EXTRACTS OF THE PLANTS SUPPLEMENTED WITH FORMULATED CEREAL AT 5% LEVEL ON YOUNG MALE ALBINO RATS AFTER FEEDING FOR 28 CONSECUTIVE DAYS BOTH FOR THE EXPERIMENT GROUP AND CONTROL GROUP.

V.A Introduction:

Dietary effects of ethanol extracts of the medicinal plants under present investigation supplemented separately with formulated cereal at 5% level have been investigated in the present study. A survey of 28 consecutive days on young male albino rats, body weight ranging from 52-56 g was performed. Changes in haematological values viz, total count (TC) of RBC and WBC, differential count (DC) of WBC, percentage of haemoglobin in the blood, erythrocyte sedimentation rate (ESR) and percentage of cholesterol in the blood of both control and experiment groups of rats were investigated to detect any abnormality that may caused during feeding the diets for a period of 28 consecutive days. Haematological investigation deals with the composition, formation, destruction, production and conservation of blood and related substances in blood , blood plasma and tissue fluids. The dietary effects of feeding the extracts, separately fortified with the formulated cereal at 5% level for a period of 28 consecutive days on the composition of red cells like erythrocytes and white cells, viz, leucocytes and platelets (thrombocytes) of the rats were investigated in this experiment. Its effects on the composition of white cells like esnophil, neutrophil, monocytes, basophil and lymphocytes of the rats under experimental and control groups were also investigated.

Histopathological as well as toxicological effects of the ethanol extracts, if any, were investigated on livers, lungs, kidneys, hearts and spleens of the experimental rats. These were achieved by observing the changes in the cellular structures of these organs. The present investigation included the identification and toxicological effects in those organs of the experimental rats. These were achieved by observing any changes in the

cellular structures (degradation and regeneration) of organs of the experiment rats after taking fortified cereal for a period of 28 consecutive days. Dietary effects of feeding on the experimental rats during the experimental period were evaluated by careful observation of the depression of central nervous system, excitation, muscular reflexes and behaviour of the rats.

V.B Materials and methods:

Potassium chloride, potassium dihydrogen phosphate, potassium iodide, sodium chloride, sodium fluoride, calcium carbonate, magnesium sulphate, magnesium carbonate, alum, copper sulphate, manganese sulphate, ferric phosphate, etc. were purchased form BDH, Poole, England. Vitamin capsules (Opsovit Capsuls, Opsonin, Bangladesh, Ltd.), Cod Liver Oil (Drug International, Bangladesh, Ltd.), Cow-ghee (Milk Vita Cow's Ghee) and full cream milk powder (Bangladesh Milk Producer's Co-operative Union Limited, Sirajganj.), barley, dried chick pea, soyabean oil ("Muskan" Pure Soabean Oil, S.A. Oil Refinery, Ltd., Chittagong, Bangladesh.), cane sugar, whole wheat flour, etc. were collected from local market. Formulated cereal in association with its salt mixture and sugar-vitamin mixture was prepared according to the method cited in the literature.[156-158] The compositions of formulated cereal, sugar-vitamin mixture and salt mixture have been shown in Table-XVII, Table-XVIIA and Table-XVIIB respectively.

For the preparation of seven experimental diets, each of the ethanol extracts of the plants was supplanted separately at 5% level with the formulated cereal. Ethanol extracts of the medicinal plants under present study were previously preserved in a refrigerator at 4^{o}C. Sixteen young male albino rats having body weights 52-56g were collected. Two of them were grouped for each of the seven diets. The diets were prepared by supplementing the extracts separately at 5% level with formulated cereal. The rats were kept in rat house in steel-wire cages. Two control rats were

simultaneously maintained only for the formulated cereal (free from plant extracts) in identical conditions. The rats were kept under proper supervision. Optimum conditions were maintained for the housing and survival of experiment rats. Consumption of food for each rat was recorded every 24 hours. Body weight was recorded on every alternate day. The experiment of feeding was carried out on the rats for a period of twenty eight days.

The data for gain in body weigh, total food intake and gain in body weight per one gram of food intake by the individual rats for feeding during the experimental period was shown in Table-XVIII. The data for gain in body weight per one gram of food intake as stated in Table-XIX was analyzed using a statistical method namely analysis of variance technique.[158(A)] The Table-XIXA showed the analysis of variance (ANOVA) table for the above results. For multiple comparisons for mean gain in body weight, a difference table was prepared (Table-XIXB). For testing the significance difference in Table-XIXB, Table-XIXC had been prepared for Duncan's New Multiple Range (DNMR) test[159] for protection level $\alpha = 0.01$. Table-XIXD was computed for DNMR test for protection level $\alpha = 0.05$.

V.C Investigation of haematological parameters:

After experimental period, the rats under investigation were killed under mild anesthesia with ether-chloroform system and their blood was collected and preserved. For histopathological investigations, the livers, lungs, kidneys, hearts and spleens of the rats were isolated, weighed and preserved for examination. Haematological profiles, like Total count (TC) of RBC and WBC, differential count (DC) of WBC, percentage of haemoglobin in the blood, erythrocyte sedimentation rate (ESR) and percentage of cholesterol in the blood of the rats were determined. The results were summarized in Table-XX. Reference values for haematological parameters[160] of the Albino rats were cited in Table-XXI. The excitation and depression of central nervous system, muscular reflexes and behaviour of the rats were carefully observed during the experiment of feeding.

V.D Histopathological investigation of the rats under study:

Histopathological investigations of the liver, lung, kidney, heart and spleen of the experimental and control rats were performed. These organs, previously preserved in a refrigerator, were separately sliced in pieces and processed in 10% formalin solution for 3 days. After proper processing and staining, these tissues were mounted on glass slides and viewed under microscope[161] having high resulation. Haematological and histopathological parameters were investigated in Rajshahi Medical Collage, Rajshahi. Microscopic investigations of sliced tissues of the organs on glass slides were summarized in Table-XXII.

V.E Results and Discussion:

Haematological and histopathological investigations of the ethanol extracts of the plants, supplemented separately with formulated cereal at 5% level on young male albino rats after feeding for 28 consecutive days both for the experimental rats and control group were performed. During this experiment, the effect of feeding the supplemented cereal on gain in body weights of the rats during the experimental period was investigated simultaneously. A control group of the rats for formulated cereal (without having any extracts) was also maintained for gain in body weight. Initial body weight of the rats, gain in body weight during the experiment of feeding and gain in body weight per one gram of food intake by each of the rats were summarized in Table-XVIII. The table resembled that there was a close similarity of the diets in gain in body weight of the rats. It appeared from the table that the diets had almost equal impact in gain in body weight. Table-XIX was computed to show the gain in body weight for one gram of food intake by 16 young male albino rats for feeding eight diets. Analysis of variance Table-XIXA and a difference Table-XIXB for multiple comparison of mean gain in body weight per one gram of food intake were computed to show which of the diets was statistically more significant for gain in body weight and for the normal development of the rats. It was evident from the tables that all the diets had similar impacts for normal development and maintenance of the rats. None of the diets was statistically significant in gain in body weight of the rats. Table-XIXC and Table-XIXD were prepared for

Dancan's New Multiple Range Test[159] for protection level $\alpha = 0.01$ and $\alpha = 0.05$. The tables failed to identify which of the diets was statistically significant at 1% or 5% levels of significance. The tables also showed that the treatments and errors of the diets had no Least Significance Range (LSR) in comparison with their respective Standard Significance Range (SSR).[162]

Total count of RBC and WBC, differential count of WBC and percentage of haemoglobin in the blood of the rats were investigated and the results were summarized in Table-XX. Comparing these values with those of reference values (Table-XXI) for haematological parameters,[160] it resembled that the changes in the parameters remained within the normal limit. No abnormalities in haematological investigations were detected. During the experimental period, the rats under investigation were found to restore no signs of muscular numbness of the legs, salivation, excitation, weakness, diarrhoea, etc. and no reflex abnormalities were detected. Thus it appeared that the dietary effects of feeding the ethanol extracts of the plants fortified at 5% level with formulated cereal for a period of 28 consecutive days had no adverse action on both control and experimental rats.

At the end of the feeding experiment for a period of 28 consecutive days, the rats were sacrificed under mild anaesthesia with ether-chloroform and their livers, lungs, kidneys, hearts and spleens were preserved, processed, sliced and the tissues were mounted on glass slides and viewed under microscope to find any histopathological change in the organ. Microscopic view of sliced tissues of the organs on glass slides were summarized in Table-XXII. On gross necropsy evaluation, all the organs under examination exhibited normal colour. No abnormal histopathological change in the cellular structure in the tissues was detected. This implied from microscopic view of those organs that the effect of feeding the ethanol extract at 5% level with the formulated cereal for 28 consecutive days had no adverse effect on cellular structure of those organs of the experiment rats and no morphological changes occurred therein.

V.F Conclusion :

From the foregoing evidences, it appeared that the ethanol extracts of the above plants, fortified with formulated cereal at 5% level was non-toxic.

Table-XVII: Composition of formulated cereal[156-158,163]

Ingredients	Amount,	Calories per 100g	Total calories present
Whole wheat flour	200g	364	728
Full cream milk powder	400g	511	2044
Starch	100g	362	362
Salt mixture (Table-XVIIA)	40g	-	-
Sugar (32.24 g) and Vitamin (7.76 g) mixture (Table-XVIIB)	40 g	385	116.42
Barley	20g	349	69.80
Dried chich pea	200g	344	688
Total	1000g	-	4008.22

Table-XVIIA: Composition of salt mixture[158]

Ingredients	Amount
Potassium chloride	112g
Potassium dihydrogen phosphate	212g
Potassium iodide	0.08g
Sodium chloride	69g
Sodium fluoride	1g
Calcium carbonate	543g
Magnesium sulphate	16g
Magnesium carbonate	25g
Alum	0.17g
Copper sulphate	0.90g
Manganese sulphate	0.35g
Ferric phosphate	20.5g
Total	1000g

Table-XVIIB: Composition of sugar-vitamin mixture.[156]

Ingredients	Amount
(a)Vitamin capsules* (106.38g) each containing the following ingredients:	106.38g
Thiamine hydrochloride, B.P.	5 mg
Riboflavin, B.P.	2mg
Pyrodoxine HCl, B.P.	2mg
Nicotinamide, B.P.	20mg
(b) Vitamins in oil** (1g) contains:	1 g
Vitamin A	700 I.U.
Vitamin D	80 I.U.
α- Tokopherol (Vitamin K)	12 mg
(c) Cane sugar	332.62 g
Total	440 g

* Opsovit Capsuls, Opsonin, Bangladesh Ltd.

** Cod liver Oil, Drug International, Bangladesh Ltd.

Table-XVIII: Supplementary effect of feeding ethanol extracts of the plants individually fortified with formulated cereal at 5% level for a period of 28 consecutive days on gain in body weight by young male albino rats having body weight 52-56g.

Nature of diets	Rat No.	Initial body weight (g)	Gain in body weight (g)	Food intake (g)	Gain in body weight per one gram food intake (g)
Diet-A Extract of *Andrographis paniculata* N. 5% + Formulated cereal 95%	1	53.28	95.26	236.13	0.403
	2	54.61	90.81	232.26	0.391
Diet-B Extract of *Vinca rosea* L. 5% + Formulated cereal 95%	1	55.32	88.56	241.32	0.367
	2	52.57	97.37	237.84	0.409
Diet-C Extract of *Adhatoda vasica* N. 5% + Formulated cereal 95%	1	56.82	86.21	231.76	0.372
	2	55.42	94.52	237.48	0.398
Diet-D Extract of *Vitex vegundo* L. 5% + Formulated cereal 95%	1	52.18	94.74	229.18	0.413
	2	53.47	87.19	234.37	0.372
Diet-E Extract of *Aloe indica* W. 5% + Formulated cereal 95%	1	54.68	95.83	228.33	0.420
	2	55.12	91.33	233.59	0.391
Diet-F Extract of *Flacortia ramontchi* 5% + Formulated cereal 95%	1	56.29	92.58	232.16	0.399
	2	53.87	99.72	236.73	0.421
Diet-G Extract of *Nyctanthes arbortristis* L. 5% + Formulated cereal 95%	1	53.32	93.33	239.88	0.389
	2	54.76	84.92	233.37	0.364
Diet-S Formulated cereal (without extracts) for control rats	1	52.55	93.88	242.59	0.387
	2	55.43	88.15	235.07	0.375

Table-XIX: Gain in body weight for one gram of food intake by 16 male albino rats for feeding eight diets for a period of 28 consecutive days.

Replications	Diet-A	Diet-B	Diet-C	Diet-D	Diet-E	Diet-F	Diet-G	Diet-S	Grand total
1	0.403	0.367	0.372	0.413	0.420	0.399	0.389	0.387	6.271
2	0.391	0.409	0.398	0.372	0.391	0.421	0.364	0.375	
Total	0.794	0.776	0.770	0.785	0.811	0.820	0.753	0.762	
Mean	0.397	0.388	0.385	0.393	0.401	0.410	0.377	0.381	

Table-XIXA: ANOVA table for gain in body weight per one gram of food intake.

Squares of variation	Degree of freedom	Sum of squares (S.S.)	Mean (S.S.)	Calculated $F = \frac{MeanS.S(Treat)}{MeanS.S(Error)}$	Tabulated F^{164}	
					5% level	1% level
Treatment	7	0.0025	0.000357	1.9091	3.50	6.18
Error	8	0.0015	0.000187			
Total	15	-				

Table-XIXB: Difference table for multiple comparison of mean gain in body weight per one gram of food intake.

Treatments in order of magnitude	Mean gain in body weight (g)	Order of the mean (K)						
		K=2	K=3	K=4	K=5	K=6	K=7	K=8
Diet-F	0.410							
Diet-E	0.401	F-E=0.009						
Diet-A	0.397	E-A=0.004	F-A=0.013					
Diet-D	0.393	A-D=0.004	E-D=0.008	F-D=0.017				
Diet-B	0.388	D-B=0.005	A-B=0.009	E-B=0.013	F-B=0.022			
Diet-C	0.385	B-C=0.003	D-C=0.008	A-C=0.012	E-C=0.016	F-C=0.025		
Diet-S	0.381	C-S=0.004	B-S=0.007	D-S=0.012	A-S=0.016	E-S=0.020	F-S=0.029	
Diet-G	0.377	S-G=0.004	C-G=0.008	B-G=0.011	D-G=0.016	A-G=0.020	E-G=0.024	F-G=0.033

Table-XIXC: DNMR Test for protection level α=0.01

Value of K=15	2	3	4	5	6	7	8
S.S.R. [162]	4.17	4.37	4.53	4.58	4.64	4.72	4.77
L.S.R.	0.0128	0.0134	0.0139	0.0140	0.0142	0.0144	0.0146

Table-XIXD: DNMR Test for protection level α=0.05

Value of K=15	2	3	4	5	6	7	8
S.S.R.[162]	3.01	3.25	3.31	3.36	3.38	3.40	3.42
L.S.R.	0.0092	0.0099	0.0101	0.0127	0.0103	0.0104	0.0105

Table: XX:- Haematological parameters of control and experimental groups of rats for feeding ethanol extracts fortified at 5% level with formulated cereal for a period of 28 consecutive days.

Erythrocyte sedimentation rate (ESR) in mm	Formulated ceteal+5% ethanol extracts of the plants	Rat No.	Haematological profiles							
			Cholesterol mg/dl	Haemoglobin g/dl	Total count		Differential count of leucocytes			
					RBC ($\times 10^6/\mu l$)	WBC ($\times 10^3/\mu l$)	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %
4.6 2.7	Diet-A	1 2	142.6 137.2	14.2 16.9	8.2 7.1	12.7 10.1	9 7	82 74	2.7 3.1	0.46 0.57
2.3 3.8	Diet-B	1 2	138.2 135.7	14.5 16.4	8.2 6.8	10.8 13.3	15 11	86 77	1.7 0.9	0.42 0.48
2.2 3.4	Diet-C	1 2	140.4 145.5	15.7 13.6	8.6 7.5	11.3 14.6	7 11	89 78	3.2 2.8	0.52 0.36
4.1 2.5	Diet-D	1 2	151.8 144.2	13.2 16.8	6.9 8.6	10.5 14.2	11 9	90 81	1.4 2.3	0.44 0.51
3.2 3.9	Diet-E	1 2	149.9 144.4	16.4 14.7	6.8 9.2	12.6 14.8	12 14	76 87	3.2 2.7	0.53 0.39
2.7 4.5	Diet-F	1 2	139.2 134.8	13.9 15.5	9.6 7.7	10.5 9.6	10 12	89 78	1.6 2.8	0.49 0.54
2.8 3.7	Diet-G	1 2	147.7 141.3	14.1 16.4	8.8 6.3	10.2 12.9	14 12	82 88	3.2 1.8	0.55 0.42
4.2 3.1	Diet-S Formulated cereal (control diet)	1 2	142.3 138.6	15.3 13.8	7.8 9.3	9.8 13.5	8 11	78 87	2.9 1.2	0.58 0.39

Table-XXI: Reference values for haematological parameters of Albino rats.[160]

Total count		Differential count of leucocytes			
RBC ($\times 10^6/\mu l$)	WBC ($\times 10^3/\mu l$)	Neutrophil (Percentage)	Lymphocytes (Percentage)	Monocytes (Percentage)	Eosinophil (Percentage)
6.26-8.98	9.4-14.9	4.5-23.3	72-94	0.5-3.5	0.35-0.60

Table-XXII: Histopathological investigation of the section of livers, lungs, kidneys, hearts and spleens of the rats of both experimental and control groups after feeding the diets for 28 consecutive days.

Groups	Livers	Lungs	Kidneys	Hearts	Spleens
Experimental Groups	No abnormality detected	No pathological changes occurred	Similar to those of control group	No Morphological changes occurred in cardiac muscle	No detectable abnormality observed
Control Groups	No abnormality detected	No abnormality detected	No abnormality detected	No abnormality detected	No abnormality detected

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Conclusion

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

CONCLUSION

Antimicrobial activities of the crude ethanol extracts of the leaves of seven bitter medicinal plants under present investigation were estimated. The extracts exhibited appreciable antimicrobial activities on four gram-positive bacteria and five gram-negative bacteria mentioned earlier at different concentrations.

The minimum inhibitory concentrations (MIC) of ethanol extracts of the leaves of the plants against the bacteria were determined by serial dilution technique and they were summarized in the table. It was evident that the MIC values of the extracts had remarkable significance about the therapeutic effects of the active principles associated with the leaves of the plants.

Brine Shrimp Lethality bioassay and cytotoxicity of the ethanol extracts were performed. The results in the tables and graphs established a positive correlation between Brine Shrimp Lethality and cytotoxicity. Brine Shrimp Lethality bioassay resembled the moderate cytotoxic effects of the ethanol extracts. The cytotoxic effects of the ethanol extracts of the plants are highly significant about their therapeutic effects.

Haematological and histopathological investigations of the ethanol extracts of the plants, supplemented separately with formulated cereal at 5% level on young male albino rats after feeding for 28 consecutive days both for the experimental rats and control group were performed. It appeared from the table that the diets had almost equal impact in gain in body weight. Statistical analysis showed that none of the diet was statistically significant in gain in body weights of the rats. Total count of RBC and WBC, differential count of WBC and percentage of haemoglobin in the blood of the rats were investigated and the results were computed in tables. The results in the tables resembled that the changes in the parameters remained within normal limit. No abnormalities in haematological investigations were detected. During the experimental period, no signs of muscular numbness of legs, salivation, excitation, weakness, diarrhoea, etc. and no reflex

abnormalities were detected. At the end of the feeding experiment for a period of 28 consecutive days, the rats were sacrificed under mild anaesthesia with ether-chloroform. Their livers, lungs, kidneys, hearts and spleens were preserved, processed, sliced and the tissues were mounted on glass slides and viewed under microscope to find any histopathological change in the organs. On gross necropsy evaluation, all the organs under examination exhibited normal colour. No abnormal histopathological change in the cellular structure in the tissues was detected and no morphological changes occurred therein. From the foregoing evidences, it appeared that the effect of feeding ethanol extracts of the above plants, fortified separately with formulated cereal at 5% level was non-toxic.

References

Andrographis paniculata **N.**

Vinca rosea **L.**

Adhatoda vasica **N.**

Vitex vegundo **L.**

Aloe indica **W.**

Flacortia ramontchi

Nyctanthes arbortristis **L.**

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