DISCUSSION
5.1 Phytochemical analysis

Phytochemical screening of the plant extracts revealed presence of most of the phytoconstituents but they were not uniformly distributed in all the extracts. Since like dissolves like, polarity of solvents is responsible for dissolving phytochemicals of similar polarity (Finar, 2003). That is why presence of all test components was not uniformly observed in different extracts. However few groups of compounds were found to be present in all extract fractions derived from test plants such as phenols and flavonoids (Table 4.3). Presence of tannins was observed in all extracts derived from *B. variegata*. Terpenoids and alkaloids were present in all sequential extracts of *P. longum* and *T. cordifolia*. Alkaloids accounted for universal presence in *P. longum* extracts. Rest of the phytoconstituents showed variable distribution. Chemical basis of their presence in different fractions may be correlated with small structural differences in the compounds belonging to same group that are critical to their activity as well as solubility (Cowan, 1999). For example flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). The addition of a 3-hydroxyl group yields a flavonol (Fessenden and Fessenden, 1982). Flavonoids are also hydroxylated phenolic substances but occur as a C6-C3 unit linked to an aromatic ring. Occasionally tannins and terpenoids will be found in the aqueous phase, but they are more often obtained by treatment with less polar solvents (Cowan, 1999).

There are reports that many active components are not water soluble, and the most commonly used solvents (ethanol and methanol) may not demonstrate the greatest sensitivity in yielding biologically active chemicals on an initial screening (Cowan, 1999). Therefore we used solvents (hexane, benzene, chloroform, ethyl acetate, acetone, ethanol and water) based on increasing polarity that led to sequential extraction of different phytoconstituents present in the same sample. Since flavonoids have been attributed with antimicrobial and free radical scavenging activities, their quantitation was done. The amount of flavonoid content in test plants was found in order of *B. variegata > T. cordifolia > P. longum*. 

Discussion

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5.2 *In Vivo* Effect of Extracts on Biomarkers in AlCl₃ Induced Hepatotoxicity

5.2.1 Effect of aqueous and ethenolic extracts on hepatic enzymes

In living systems, liver is considered to be highly sensitive to toxic agents. Biochemical and enzymological profile of blood are widely used as diagnostic markers for the assessment of liver function status. The study of total bilirubin, creatinine and enzymes activities serum glutamate oxalate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP) in blood have been found to be of great value in the evaluation of clinical and experimental liver damage (Vaishwanar, 1976). These parameters also help in monitoring the degree of protection provided by drugs/test plant extracts. The aim of the present study was to investigate the effects of administering doses of aluminium in the form of AlCl₃ on the hepatotoxic and oxidative stress indices in the rat liver, and their modulation by plant extracts.

Major hepatotoxic effects of aluminium toxicity were observed on GOT and GPT activities. About 2.5 fold increase in GOT activity (about 200 U/L) was found as compared to the normal range (45-80 U/L). Co-administration of Liv-52 (standard drug) with aluminium accounted for bringing down the altered enzyme level to normal. Administration of all test plant extracts with aluminium caused marginal drop in GOT activities but it was still beyond range (about 160-180 U/L) (Figure 4.1- 4.4). Metal was also responsible for causing 45% elevation of GPT level in rat serum (normal range 17-30 U/L). *B. variegata* AQ and *T. cordifolia* ET extracts accounted for lowering the GPT activities to near normal level indicating hepatoprotective response. Similar result was also observed with Liv-52. Other extracts also showed potential but slightly lower efficacy. Control groups showed normal ALP values but on the lower side of the standard range (56-128 U/L). Aluminium caused elevation in enzyme activity but it was still in the normal range. Among test extracts *T. cordifolia* showed greater hepatoprotective potency than other two plants extracts. *T. cordifolia* is also used as liver protectant in Ayurveda (Mishra et al., 2013). Hence our results provide scientific basis to the usage of this plant products as hepatoprotectant.
5.2.2 Effect of aqueous and ethenolic extracts on other serum biomarkers

Aluminium was responsible for increasing the serum total bilirubin level up to 0.72 mg/dl which is about 40% higher than normal value (0.2-0.55 mg/dl). Liv-52 was effective in lowering down the the levels to normal range. Test extracts could not produce significant effect (0.62-0.66 mg/dl). Similar trend was observed with direct bilirubin content. Marginal increase in creatinine content was observed under influence of metal (Figure 4.5 and 4.6). Most of the extracts and Liv-52 exhibited creatinine lowering effect and brought down the values towards normal range (0.2-0.8 mg/dl). *T. cordifolia* aqueous extract showed maximum creatinine lowering effect (0.64 mg/dl). In general plant extracts showed hepatoprotective effect against aluminium induced toxicity.

5.2.3 Effect of extracts on antioxidant enzymes in tissue homogenate

The enzymatic antioxidant defense system such as Superoxide dismutase (SOD) and catalase (CAT) serves as a first line of defense against oxidative stress and protect the cellular constituents against oxidative damage. SOD is extensively used as a biochemical indicator in pathological condition associated with oxidative stress (Patra et al., 1998). The toxic effect of metal is mediated by free radical generation which results in the oxidative deterioration of cellular lipids, proteins and DNA and also induces changes in the number of tissue antioxidant enzymes (SOD and CAT) (Moumen et al., 2001).

In present study AlCl$_3$ (100mg/kg bw) was found to cause noticeable oxidative stress in rats as indicated by decreased activity of SOD and CAT (Figure 4.7 and 4.8) in liver, kidney and brain tissues. Several report have shown that aluminum causes increase in lipid peroxidation (LPO) and inhibition of antioxidant enzymes in plasma, brain, testes, kidney, renal cortex, serum, erythrocytes, and liver (Mohammadirad and Abdollahi, 2011). Co-administration of plant extracts helped in substantial restoration of antioxidant enzymes. *T. cordifolia* extracts showed pronounced antioxidant response. The aluminium toxicity is generated by production of reactive oxygen species (ROS) which may be due to their “redox cycling” activity. They readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction. If continuous supply of antioxidants is maintained, it minimizes oxidative stress and thereby resulting in normal homeostasis of body (Vidyasagar et al., 2004).
The protection offered by the test extracts (TC- AQ, ET; PL- AQ; BV- AQ) could have been due to the presence of various phytoconstituents including flavonoids and alkaloids. Thus, the extracts activate the antioxidant enzymes and thereby protect the tissues from oxidative damage induced by aluminum chloride. Alkaloids have been reported to strongly inhibit LPO induced in isolated tissues through its antioxidant activity (Kumaran et al., 2007). Our results are in agreement with these findings. Plants are the essential and integral part in treatment strategy against metal toxicity as they form secondary metabolites such as proteins, flavonoids, alkaloids, steroids, and phenolics which in turn are used to restore health and heal many diseases. The cell has developed an intricately regulated antioxidant defence system to protect against toxic effects of ROS and to modulate physiological effects of ROS. Antioxidant enzymes such as superoxide dismutase SOD metabolizes the superoxide anion into peroxide, and catalase CAT catalyses the decomposition of H$_2$O$_2$ into water and O$_2$ (Halliwell and Gutteridge, 1999). Imbalance between oxidant and antioxidants causes oxidative stress, which is a major contributing factor in several human chronic diseases, such as atherosclerosis, cardiovascular diseases, several neurodegenerative disorders, mutagenesis and cancer, and probably the ageing processes (Halliwell and Gutteridge, 1999).

5.3 Assessment of Antioxidant Power of Extracts in vitro

5.3.1 β-carotene bleaching assay

Chromophores such as β-carotene have alternate double and single carbon-carbon bonds which are known as conjugated system. The electrons in the π-orbitals of the double bonds overlap, creating a system of delocalized electrons across a large part of the molecule. Carotenoids undergo bleaching (loss of color) when exposed to radicals or to oxidizing species which involves interruption of the conjugated double bond system either by cleavage or by addition to one of the double bonds (Huang et al., 2005). The results demonstrated that some of B. variegata leaf extracts (BZ, CH and AQ), T. cordifolia stem extracts (CH, ET and AQ) and P. longum fruit extracts (BZ, EA and AC) possessed appreciable β-carotene bleaching inhibition, activity (ZOI > 15mm). The antioxidant action shown by these extracts could be attributed to their radical scavenging activity (Sharma et al., 2014b). Polyphenolic contents of the extracts have been reported to function as good electron and hydrogen atom donors and therefore they have ability to terminate radical chain reaction by converting free
radicals and ROS to more stable products (Mishra et al., 2013b). Therefore presence of phenol, flavonoids, alkaloids and other phytoconstituents in test extracts are responsible for mediating antioxidant activity.

5.3.2 FRAP assay

Antioxidant activity is one of the important pharmacological activities present in plants. FRAP assay uses a complex of ferric ion and TPTZ as reagents to assess antioxidant capacity of the test samples. The FRAP assay relies on the reductive ability of the antioxidant compounds by facilitating reduction of the ferric ion-tripyridyltriazine (TPTZ) complex to the ferrous ion-TPTZ complex which is measured at 593 nm (Benzie and Strain, 1996; Luximon et al. 2002). This gives a direct measurement of antioxidant capacity of samples. The results demonstrated that B. variegata leaf (EA, AQ, AC and ET), T. cordifolia stem (CH, EA, AC and ET) and P. longum fruit (AC) extracts have higher FRAP values indicating the potent antioxidant capability of the phytoconstituents present in these test samples. Secondary metabolites especially flavonoids have been reported to act as reducing agents (Kumar and Pandey, 2013). Our group has earlier reported a positive correlation between total flavonoid content and reducing power of the B. variegata leaf extracts (Mishra et al., 2013). Hence higher FRAP values observed in the study could be attributed to the reducing power and the flavonoid content of the extracts.

5.3.3 Anti-hemolytic activity

Hemolysis has long been used to measure free radical damage and its inhibition by antioxidant compounds in whole blood erythrocytes provides basis for selection of potential antioxidants. This assay is useful for screening of various chemical compounds and plant extracts having antioxidant potential (Djeridane et al., 2007). In present study we used rat blood erythrocytes were used to study H2O2 induced membrane damage. Lipid oxidation of rat blood erythrocyte membrane mediated by H2O2 induces membrane damage and subsequently hemolysis occurs. Most of the T. caordofolia stem and P. longum fruit extracts showed moderate anti-hemolytic activity (Figures 4.11 A–C). EA fraction of P. longum fruit exhibited about 64% anti-hemolytic activity (Figure 4.11). HX and AQ fraction of B. variegata leaf exhibited moderate anti-hemolytic activity (56% and 43%, respectively). Several studies have revealed moderate to higher efficacy of plant products against lipid oxidation in the erythrocytes membranes (Djeridane et al., 2007; Zhu et al., 2002).
5.3.4 Lipid Peroxidation Inhibition (LPOI)

In mammals lipid peroxidation is related to injury and inflammation and is often due to the oxidative deterioration of the cellular membrane lipids. Peroxidation of lipids in the body may be enzymatic, non-enzymatic or both. Non enzymatic reaction involves initiation, propagation and termination phases (Gueraud et al., 2010). Lipid, lipoperoxyl, lipid hydroperoxide, peroxy and alcoxy radicals produced in the first two phases of lipid peroxidation are deleterious to the body. Malondialdehyde (MDA), an important byproduct of the lipid peroxidation, reacts with thiobarbituric acid (TBA) to form TBA-MDA adduct with an absorbance maximum at 532 nm (Kumar et al. 2013a). B. variegata leaf (AQ), T. cordifolia stem (ET) and most of the P. longum fruit extracts exhibited significant lipoprotective activity against iron induced membrane damage in rat liver homogenate as indicated by diminution in adducts formation (Figure 4.12). Our findings are in agreement with the other reports which have also revealed the protective effect of plants against membrane damage (Kumar and Pandey, 2014; Gulcin et al., 2010). The antioxidant activity could be attributed to the metal ion chelating and/or free radical scavenging activities of the phytochemicals present in the test extracts (Kumar et al. 2014; Yen et al. 1993). In the present study most of the test extracts showed significant presence of flavonoid content (Table 4.4). Other studies on lipoprotective efficacy of flavonoid also corroborated our findings as they demonstrated a positive correlation between these two parameters (Kumar and Pandey, 2014; Gulcin et al., 2010). Available reports tend to show that secondary metabolites of phenolic nature including flavonoids are responsible for the variety of pharmacological activities (Kumar and Pandey, 2013). Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants which might be due to their phenolic compounds, specifically to flavonoids (Pourmorad et al., 2006). Flavonoids are a group of natural compounds with variable phenolic structures found ubiquitously in plants. The pharmacological activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. Lipid peroxidation is a common consequence of oxidative stress. Flavanoid protect lipids against oxidative damage by various mechanisms. Because of their capacity to chelate metal ions, flavonoids also inhibit free radical generation (Kumar and Pandey, 2013). Several flavonoids such as catechin, apigenin, quercetin, naringenin, rutin and venoruton are also reported for their hepato-protective activities (Pourmorad et al., 2006).
5.4 Anticancer Activity of Plants Extracts

5.4.1 Assessment of cytotoxic potential of extracts

Medicinal plants have played a major role in the development of modern medicine and continue to be widely used in their original form. Significant progress has been made over the past two decades in understanding the molecular and cellular mechanisms of pre-cancer and cancer progression. Nonetheless, the development of effective and safe agents for prevention and treatment of cancer remains slow, inefficient and costly, with little to offer the high risk population for primary cancer prevention and cancer survivors to prevent cancer recurrence. The key to effective chemotherapy and chemoprevention is the identification of chemotherapeutic and chemopreventive agents that can effectively inhibit cancer development without toxic side effects (Zou et al., 2005).

The use of plant products for treatment of cancer is another alternative. Many plant-derived compounds have been an important source of several clinically useful anticancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide derived from epipodophyllotoxin, and paclitaxel (Cargg et al., 2005). Anticancer agents having low side effects, inducing apoptosis and target specific cytotoxicity are drug of choice (Skehan et al., 1990). Keeping this in mind we investigated the cytotoxic activity of extracts of *B. variegata*, *T. cordifolia* and *P. longum* against human cancer cell lines.

In the present study aqueous extracts derived from *B. variegata*, *T. cordifolia* and *P. longum* exhibited effective cytotoxic potential against Colo-205, HCT-116 and A549 cell lines (Figure 4.13, 4.14 and 4.16). In addition some non polar fractions (HX, BZ, AC) of *P. longum* also produced remarkable inhibition (>90%) of A549 lung cancer cell lines (Figure 4.15). The variable response of cell lines towards extracts might be due to differential molecular characteristics of these cells. Moreover, extracts derived from test plants also showed difference in phytochemical constituents at qualitative and quantitative levels. The results are in agreement with the reports on *Polyalthia longifolia* (Verma et al., 2008) and *Ocimum basilicum* (Manosroi et al., 2006). The study demonstrated that *B. variegata*, *T. cordifolia* and *P. longum* extracts are active against a few selected human cancer cell lines. The anticancer activity of plant products have been attributed to the presence of natural compounds. *Eucalyptus*
*citriodora* has been reported to contain 60% of the monoterpenoid citronellal, citronelol, p- cymene, limonene and n-hexyl acetate which are responsible for its anti cancer activity (Low et al., 1974). There are reports indicating biological interactions of flavonoids, polyphenols or phenolic compounds with proteins, enzymes and other biological processes in the cells that make them toxic to the cell or serve as growth inhibitors (Kumar and Pandey, 2013).

Preliminary phytochemical screening of extracts of all the test plants showed the presence of alkaloid, flavanoids, terpenoids, tannins and phenolic compounds. Flavonoids have been shown to possess antimutagenic and antimalignant effects (Brown, 1980; Hirano et al., 1989). Moreover, flavonoids have a chemopreventive role in cancer through their effects on signal transduction in cell proliferation (Weber et al., 1996) and angiogenesis (Fotsis et al., 1997). The cytotoxic and antitumor properties of the extract might be due to the presence of these compounds (Saluja et al., 2010). Further investigations are required for isolating active constituent responsible for the cytotoxicity.

*B. variegata* stem flavonoids have been shown to possess cytotoxic activity against Dalton’s ascetic lymphoma, leukemia, breast, lung (HOP-62), ovary (IGR-OV-1), prostate (DU-145) and many more cancer cell lines (Rajkapoor et al., 2003; Rajkapoor et al., 2009). It has been reported that *B. variegata* contains flavones which are more selective against OVCAR-5 and SKOV-3 cell lines of ovarian cancer showing average 50% cytotoxic activity (Rajkapoor, 2009).

*T. cordifolia* methanolic and aqueous extracts have been reported to exhibit antineoplastic activity against HeLa cell lines (Sinha et al., 2004). *T. cordifolia* extracts have also been reported to stimulate the immunity in natural as well as tumor bearing animals. Administration of *T. cordifolia* stem methanolic extract in balb/c mice has been shown to increase the total white blood cell count, bone marrow cellularity and alpha-esterase positive cells in bone marrow indicating increased maturation of stem cells. Extracts reduced solid tumour growth and synergistically acted with cyclophosphamide in reducing the animal tumours (Mathew and Kuttan, 1999). This indicates appreciable immunostimulatory and antitumor effect in *T. cordifolia* alcoholic extract *in vivo*. These immunostimulating properties can be used in the prevention of tumour mediated immunosuppression and hence could be a drug.
choice for various cancers (Balachandran and Govindarajan, 2005). Further *T. cordifolia* extracts have also been shown to alleviate the immunosuppression induced by chemo and radiotherapy (Sunila and Kuttan, 2004).

Some non polar extracts (HX and BZ) of *P. longum* also displayed noticeable cytotoxic activity against other cell lines viz., ovary (IGR-OV-1), prostrate (DU-145), breast (MCF-7) and leukemia (THP-1) cell lines (Figure 4.15). These observations indicate that presence of specific non polar chemical moieties extracted in non polar extracts could contribute to *invitro* cytotoxicity. *P. longum* alkaloids especially piperine has been shown to be cytotoxic towards MCF-7, breast cancer cell line (Intouch, 2009). The phytochemical investigations of *P. longum* fruit extracts have also shown the presence of terpenoids along with flavonoids, alkaloids and other phytochemicals (Table 4.3) in the extracts. Hence the observed antitumor property of *P. longum* fruit extracts could be attributed to the presence of various phytochemicals including steroidal and triterpinoidal molecules in the extracts. Comparison with growth inhibition activities of standard anticancer drugs (paclitaxel, mitomycin-c, adriamycin and 5-fluorouracil) against test cell lines in the present study also demonstrated significant anticancer potential in all test extract including *P. longum* extracts. Our results are in agreement with the report that petroleum ether and chloroform extracts of *P. longum* fruits significantly increase the life span of tumor (EAC cells) bearing animals (Hullatti et al., 2006). Alkaloid amides Piplartine \{5,6-dihydro-1-(1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl) 2 (1H) pyridinone\} and piperine \{1-5-(1,3)-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl\} piperidine} isolated from *P. longum* have been reported to show cytotoxic activity towards several tumor cell lines (Tsai et al., 2005). Therefore cytotoxic activity in *P. longum* could be attributed to the presence of terpenoids, steroids, alkaloids and flavonoids.

Dysregulation of apoptosis is the hallmark of all cancer cells, and the compounds that are capable of inducing apoptosis and interfering with cell cycle regulation are considered to be important in anticancer therapeutics. Activation of apoptosis pathways is a key mechanism by which cytotoxic drugs kill tumor cells. Also chemotherapy of tumors requires an apoptosis sensitive phenotype of target cells (Debatin, 2004). Food supplements containing specific dietary factors of plant origin have been shown to alter the induction of cancers in a variety of tissues and organs due to interference with cell cycle regulation (Jose et al., 2001). Based on preliminary
screening data we further studied the mechanism of cytotoxic action of potential *P. longum* extracts (HX, BZ and AC) against lung cancer cell line (A549) using cell cycle analysis. Present study revealed that treatment of A549 cells with *P. longum* extracts induced arrest of these cells in sub G1 phase of cell cycle. HX, BZ and AC extracts exhibite appreciable increase in subG1 population 41%, 63% and 43%, respectively. Arrest of cells at check points of cell cycle has been reported as an event preceding the apoptosis in the cell (Lam et al., 2012). From the present findings it is inferred that cell cycle arrest is the major mechanism behind cytotoxic activity of *P. longum* extracts.

Various stresss including DNA damage induce cell cycle arrest. In response to DNA damage, check points arrest the cell cycle in order to provide time for DNA repair. DNA damage check points are positioned before the cell enters S phase (G1-S check point) or after DNA replication (G2-M check point) and there appears to be DNA damage check point during S and M phases. G1, S, G2 and M are traditional subdivisions of the standard cell cycle. Cells in G1 can, before commitment to DNA replication, enter a resting state called G0. Cells in G0 account for the major part of the non-growing and non proliferating cells in human body. Targeting the cell cycle in general and cyclin-dependent kinases (CDK) in particular present unique opportunities for drug discovery (Vermeulen et al., 2003). Our results clearly indicate the anti-cancer potential in test plants. Further detailed work on these extracts can provide us with an effective non-toxic anti tumor agents.

**5.5 Anti-Infective Activities of Extracts**

**5.5.1 Antibacterial activity of plant extracts**

Medicinal plants have been used since ages for the treatment of diseases (Penalver et al., 2005). Plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts, showing great potential especially against multidrug resistant bacteria (Mishra et al., 2013a; Mishra et al., 2013b). In recent years, herbal medicines have increasingly been used to treat infections difficult to manage (Lin et al, 2005). Phytochemicals are important sources of naturally occurring potentially useful chemical moieties for the development of new chemotherapeutic agents. Many reports are available on the antibacterial, antifungal and antiviral properties of plants (Behera and Misra, 2005; Bylka et al., 2004; Govindarajan et al., 2005; Kumaraswamy et al., 2002; Palombo and Semple, 2001).
The inhibition produced by the plant extracts against particular organism depends upon various extrinsic and intrinsic parameters. Polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. In addition differential diffusibility of extracts in agar medium is also responsible for producing variable response. Emergence of drug resistance in bacteria has given way to search for new antimicrobial substances from alternative sources including plants (Basile et al., 1999 and Pesewu et al., 2008). In the present study the plant extracts exhibited moderate antibacterial activities (Tables 4.6, 4.7, 4.8) as represented by zone of inhibition against the test bacteria. Gram positive microorganisms *S. mutans* (MTCC497), *B. cereus* (MTCC6840) and *B. bronchiseptica* (MTCC6838) showed susceptibility to many extracts derived from *B. variegata, T. cordifolia* and *P. longum* (HX, BZ, CH, EA and AQ). However *B. cereus* exhibited lower susceptibility to most of the extracts derived from *T. cordifolia*.

In general, among the tested microorganisms, some of the bacteria were found to be moderately sensitive to many of the test extracts. The antibacterial activity was more pronounced on Gram-positive bacteria than Gram negative bacteria. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria might be attributed to the difference in morphological constitution between these microorganisms. Gram negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The Gram-positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram negative bacteria which are more complex than Gram-positive bacteria act as a diffusion barrier and making them less susceptible to antimicrobial agents than Gram-positive bacteria (Hodges, 2002). In spite of this permeability differences, many of the extracts still exerted some degree of inhibition against Gram negative bacteria as well.

Secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins and other compounds of phenolic nature are responsible for the antimicrobial activities in higher plants (Cordell, 2001). Monoterpenes, sesquiterpenes, alcohols and aldehydes have been reported to exhibit antibacterial activity in spices against respiratory tract infections (Inouye et al., 2001; Skocibusic, 2004; Viljoen, 2005). Cyclic terpene compounds have been reported to cause loss of membrane integrity and dissipation of
proton motive force (Sikkema, 1995). Therefore, presence of some of these phytochemicals in the extracts could to some extent substantiate the observed antibacterial activities in the present study.

Polyphenols, such as tannins and flavonoids, are important antibacterial substances. Tannins present in plants cells are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties. Many plants contain non toxic glycosides which can get hydrolyzed to release phenolics which are toxic to microbial pathogens (Aboaba and Efuwape, 2001).

The medicinal plants like *T. cordifolia*, *B. variegata* and *P. longum* are being used traditionally for the treatment of antispasmodic, anti-inflammatory, anti-allergic, anti-diabetic, bronchitis, leprosy, cough, tumors, asthma, cold, as counter-irritant and analgesic (Kavitha et al., 2011; McCune and Johns, 2002; Youdim and Joseph, 2001). Preliminary phytochemical analysis of all extract revealed the presence of alkaloids, terpenes, phenol, tannins, and flavonoids (Tables 4.1-4.3). The inhibitory effects of these plants on the test microorganisms may therefore, be due to the presence of the above phytochemical components. It has been reported that the antibacterial activity of piper was probably due to their major phytochemicals including alkaloids and related compounds. Piperine, the most abundant alkaloid in the fruits of *P. longum* is responsible for the pungency of long pepper and has been shown to possess anti-inflammatory, antiamoebic, antiasthmatic, anticonvulsant and antibacterial activities (Aneja et al., 2010). Thus, the antibacterial activity of the fruits of *P. longum* observed in the experiments may be due to the presence of the alkaloid piperine. These findings support the traditional knowledge of local users and it is a preliminary scientific validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources.

### 5.7 Antiparasitic Activity

#### 5.7.1 Antimalariais activity

Malaria is still the most destructive and dangerous parasitic infection in many tropical and subtropical countries. Multidrug-resistant *Plasmodium falciparum* strains are an increasing problem in endemic areas and are partly responsible for the worsening
malaria situation around the world. New inexpensive and effective compounds active in combination with available drug in the field are urgently needed. Recently progress in chemical analysis presented evidence that complex molecules elaborated by natural organisms could hardly be synthesized by chemical processes. Moreover, the resistance of *P. falciparum* to chemical treatment still remains important. Therefore natural products isolated from plants used in traditional medicine, which have shown potent antiplasmodial action *in vitro*, represent potential sources of new antimalarials drugs (Gasquest et al. 1993; Wright and Phillipson, 1990). Our results demonstrated that *B. variegata* (CH, BZ and EA), *T. cordifolia* (AQ) and *P. longum* (HX, BZ and CH) extracts possess substantial anti-plasmodial activity against a chloroquine-sensitive (3D7) strain of *P. falciparum* (Table 4.9). Since piperine, an alkaloid, is major constituent of *P. longum*, hence its activity against leishmania is in agreement with earlier reports. The inhibition potential of these extracts on the growth of parasite might be attributed to the presence of phytochemicals such as flavonoid content. In *B. variegata* extracts flavonoid content was found to be the highest among test samples and most of *B. variegata* extracts exhibited appreciable anti-plasmodial potential. Hence flavonoid contents in these extracts might correlate well with their inhibitory potential. This might be responsible for that assist maximum inhibitory potential against malarial parasite. A number of reports have demonstrated growth inhibitory effects of flavonoids, particularly of the flavonols quercetin and of the flavone luteolin, on the protozoan parasite genera *Toxoplasma* (Weiss et al., 1998), *Trypanosoma* (Mamani-Matsuda et al., 2004). Lehane and Saliba (2008) have also reported the effects of a range of common dietary flavonoids on the growth of two strains of *P. falciparum*. The chemical composition of many plants reported in literature indicated the presence of terpenes, euphorbol derivatives, saponins and phenolic compounds such as anthocyanins and flavonoids such as quercetin (Bakana, 1984; Oliver-Bever, 1986). Presence of flavonoids in *B. variegata* and combination of flavonoids along with other phytoconstituents in *P. longum* and *T. cordifolia* could be considered as the active constituents because of their antiplasmodial activity earlier reported (Paulo et al., 1995; Cimanga, 1997).

A tridimensional structure of a protein is essential for describing its biological functions. The detailed knowledge of structural organization is crucial in understanding the role of proteins in the cell and related molecular mechanisms. The experimental methods such as x-ray crystallography and nuclear magnetic resonance are very powerful for determining the 3D structure of biomolecules (Flores et al.,
However, these techniques have limits, being very costly, time consuming and tedious. The computational methods such as homology modeling (or comparative modeling) have proved as a powerful method for predicting the 3D structure of proteins being very fast and convenient. Which are mainly used for secondary and tertiary structure predictions of protein (Flores et al., 1993; Sali and Blundell, 1993).

In this study, a homology model of PfTk is discussed, with the aim to study structure-function relationship as well as to explore the structural similarity of PfTk with existing transketolase. The α-helix and β-content of PfTk was determined by homology model predictions. This model was used to study the binding properties of phytochemical present in P. longum viz., (pipernonaline (ZINC14658236), piperine (ZINC01529772), piperlongumine (ZINC00058185), pellitorine (ZINC13838505), piperundecalidine (ZINC14658375), dehydropipernonaline (ZINC01529277), piperettine (ZINC14657880)) by docking the ligand in the cofactor and substrate binding site of PfTk.

Docking studies not only provide an understanding of the binding mode of ligands but also are also employed to validate homology model (Singh et al., 2007). Molecular docking can fit molecules together in a favorable conformation to form a complex system. The key characteristic of a good docking program is its ability to reproduce the experimental binding mode of ligands.

Virtual screening is an emerging technology that is gaining an increased role in the drug discovery process (Abagyan and Totrov, 2001; Bissantz et al., 2000). The technology involves analyzing large collection of compounds and leading it to smaller subsets for biological testing. It is now perceived as a complementary approach to experimental screening (High throughput screening) and when coupled with structural biology promises to enhance the probability of success in the lead identification stage of drug discovery process. Structure based virtual screening requires computational fitting of compound in to an active site of a receptor by use of sophisticated algorithm, followed by scoring and ranking to these compounds to identify potential leads. Moreover, identification of these novel and phytochemically diverse inhibitors provides initial leads for optimization into more potent and efficacious drug candidates to treat malarial infection. In the present study docking of P. longum phytochemical at active site of PfTK provides mechanistic support to antimalarials activity of extracts.
5.7.2. Anti-leishmanial activity

The results shown in table 4.12 indicated that non-polar extracts of BV had appreciable antileishmanial activity of plants extract against L. major promastigotes (IC50 value 10-16 mg/ml). Available literature suggests that many phytochemicals have antileishmanial potential e.g., flavonoid and alkaloids. Piperine, which is found in many piper species, has been shown to be active against promastigotes of L. donovani with activity comparable to pentamidine (Kapil, 1993). The antileishmanial activity observed in B. variegata could mainly be attributed to the flavonoid contents in addition to other phytoconstituents. Growth inhibitory effects of flavonoids, particularly of the flavonols quercetin and of the flavone luteolin, on the protozoan parasite genera Toxoplasma, Trypanosoma and Leishmania have been demonstrated by several workers (Weiss et al., 1998; Mamani-Matsuda et al., 2004; Sen et al., 2005). Alkaloid berberine from Berberis aristata also shows significant antileishmanial activity with IC 50 (10 µl/ml) against L. major (Ghosh et al., 1985). Besides these compounds, Picroliv a standardized mixture of iridoid glycosides prepared from the root and rhizome extract of Picrorrhiza kurroa shows a significant antileishmanial activity and it is used in combination therapy of kala azar with sodium stibgluconate. It is reported to enhance the efficacy of the antileishmanial drug and also to reduce its side effects (Puri, 1992). The antileishmanial activity of plant extracts has also been attributed to compounds belonging to diverse chemical groups, such as isoquinoline alkaloids, indole alkaloids, quinones, and terpenes (Araújo et al., 1998). Plant products have also been reported to inhibit growth of other stages in the life cycle of L. major (De Queiroz, 2014).

There is lack of effective and inexpensive chemotherapeutic agents for treatment of Leishmaniasis. Although trivalent antimonial (Sb (III)) like potassium antimonyl tartrate and pentavalent antimonial drugs are the first-line treatment for leishmaniasis, with amphotericin B and pentamidine being used as alternative drugs. Growing resistance to drugs has become a severe problem. All of these drugs have serious side effects, longer length of treatment, high toxicity, and high cost. Therefore, new drugs are urgently required. Natural products offer an unlimited source of chemical diversity to identify new drug modules for the treatment of important tropical diseases caused by protozoans (Wright et al., 1990). The scientific evaluation of medicinal plants used
in the preparation of folk remedies has provided modern medicine with several effective pharmaceuticals for the treatment of diseases caused by protozoan parasites (Phillipson and Wright, 1991; Chan-Bacab, 2001).

The bioactive phytocompounds present in the plant derivatives including the crude extracts, essential oils, and other useful compounds can be a good source for discovering and producing new antileishmanial medicines. Based on the activity (IC50), potential test extracts could be considered as promising candidates for further purification and bioevaluation.