Plants contain enormous number of natural compounds with important pharmacological properties and their extracts have been used for treating various diseases from ancient times. These natural molecules have revolutionized the medicinal system (Koehn and Carter, 2005; Mishra and Tiwari, 2011). Phytomedicines continue to play a central role in health management systems in developing countries which include 65% of the Indian population. In the USA, sale of phytomedicines has sharply increased between 1988 and 1997 (WHO, 2002).

Recent World Health Organization review estimates that 80% of World’s population depends on traditional medicines (WHO, 2011). Since phytomedicine has globally been the matter of interest in primary source of healthcare (Farnsworth and Morris, 1976) that encouraged its utilization as a source of chemical diversity in drug development. Plant-derived molecule structures are known to have evolved under evolutionary pressure with diverse properties that make them suitable as lead structures in drug discovery (Evans et al. 1988). These molecules have also been recognized to provide specific substructures or scaffolds that make them comparable to trade drugs and their potential utilization in combinatorial chemistry (Basmadjian et al. 2014). Such exceptional properties exhibited by the plant derived molecules make their direct use in drug discovery as well as by using them as scaffolds to synthesize combinatorial repertoire proficient enough to bind against wide range of disease-specific targets. In fact, it could be argued that plants with medicinal values may have co-evolved with humans. Various disease treatments have become dependent now upon natural products importantly diabetes (Hung et al. 2012) and cancer (Basmadjian et al. 2014).

Despite the advent of combinatorial chemistry and high throughput screening campaigns during the last decades, the impact of natural products for drug discovery is still very high. Of the 1,073 new chemical entities belonging to the group of small molecules that had been approved between 1981 and 2010, only 36% were purely synthetic, while more than the half were derived
or inspired from nature (Newman and Cragg, 2012). A substantial number of these compounds have been discovered in higher plants (Kinghorn et al. 2011). Particularly prominent examples of plant-derived natural compounds that have become indispensable for modern pharmacotherapy can be found in the field of anti-cancer agents e.g. paclitaxel and its derivatives from yew (Taxus) species, vincristine and vinblastine from Madagascar periwinkle (Catharanthus roseus), and camptothecin and its analogs initially discovered in the Chinese tree Camptotheca acuminata (Kinghorn et al. 2011; Cragg and Newman, 2013). Further notable examples include the cholinesterase inhibitor galanthamine that has been approved for the treatment of Alzheimer's disease and was initially discovered in Galanthus nivalis L. (Mashkovsky and Kruglikova-Lvova, 1951) and the important anti-malarial and potential anti-cancer agent artemisinin originally derived from the traditional Chinese herb Artemisia annua L. (Klayman et al. 1984).

Important challenges related with the use of plants as a source for identification of bioactive compounds are related with the accessibility of the starting material. Often the available amount of natural products is low. Although many plant-derived natural products have already been isolated and characterized, available compound quantities are often insufficient for testing for a wide range of biological activities. While small amounts of plant material are usually required for an initial pharmacological evaluation, much larger quantities are needed for through characterization of the pharmacological activity of its constituents. Furthermore, limited availability becomes even more problematic when a bioactive plant derived natural product is identified to have a very promising bioactivity and becomes a pharmaceutical lead.

Besides the accessibility of the plant material, also its quality is of great importance. Available plant material often varies on quality and composition and this can hamper the assessment of its therapeutic claims. The chemical composition is not only dependent on species identity and harvest time, but also on soil composition, altitude, actual climate, processing and storage.
conditions. Moreover, during extraction, as well as during the isolation processes, transformation and degradation of compounds can occur (Jones and Kinghorn, 2012; Bucar et al. 2013). Another aspect determining the chemical composition of the starting plant material is that endophytic organisms, such as fungi and bacteria, might inhabit plants. As a result, natural products present in the collected plant material might be in some occasions metabolites of the endophytic organism, or plant products induced as a result of the interaction with this organism (David et al. 2015).

PHYTOCHEMICAL ANALYSIS

In spite of tremendous developments in the field of allopathy during the 20th century, plants still remain as one of the major source of drugs in modern as well as traditional systems of medicine throughout the World. The therapeutic properties of the medicinal plants are due to the occurrence of active principles, which has to be extracted and screened for medicinal properties. Natural products have played an important role as new chemical entities (NCEs) approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived. Another 20% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman et al. 2003). Combining these categories, research on natural products accounts for approximately 48% of the NCEs reported from 1981–2002.

Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereo centers that can be challenging synthetically (Clardy and Walsh, 2004; Nicolaou and Snyder, 2004; Peterson and Overman, 2004; Koehn and Carter, 2005). Many structural features common to natural products (e.g. chiral centers, aromatic rings, complex ring systems, degree of molecule saturation and number and ratio of hetero atoms) have been shown to be highly relevant to drug discovery efforts (Lee and Schneider, 2001; Feher and Schmidt, 2003; Clardy and Walsh, 2004; Piggott and Karuso, 2004; Koehn and Carter, 2005). Furthermore, drugs
derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists (Kingston, 2011; Scannell et al. 2012; David et al. 2015).

The present research programme on phytochemical investigations of Morus alba, Morus serrata and Morus laevigata have yielded interesting results. The phytochemical analysis revealed the presence of phenols in the petroleum ether extract of Morus alba, Morus serrata and Morus laevigata along with tannins in Morus alba and Morus serrata, while their chloroform extracts showed the presence of steroids, terpenoids, alkaloids, phenols, tannins, glycosides. However, saponins were present only in Morus alba and Morus laevigata in the said extract. Further, the methanol extract of all the species showed the presence of steroids, terpenoids, alkaloids, flavonoids, tannins, glycosides and saponins.

Total of four compounds, AD5, AD6, AD11 and AD13 were isolated from the different solvent extracts of three mulberry species using column chromatography. The structures of the isolated compounds were determined using IR, 1H-NMR and Mass spectral analyses.

**Compound 1-AD5:** The results of infrared spectroscopy revealed a strong absorbance frequency at 3412cm\(^{-1}\) observed for the –OH groups and strong absorbing frequency at 2920cm\(^{-1}\) for the alkyl group. A strong absorbance frequency observed at 1464cm\(^{-1}\) is for the C=C stretching. The results of proton nuclear magnetic resonance revealed the presence of OH group indicated by the peak appearing at the region of 5.3δ value. The multiplets for CH\(_2\) protons are appeared at the region of 1.5-1.6δ values. The peaks appearing at the region of 0.8-1.0 δ value is for -CH\(_3\) protons. The peak observed at Mass peak 291 (positive mode) confirms the molecular mass of the compound as 5-androstenediol.

5-Androstenediol (Pubchem ID:CID223407), a 3-hydroxylated C19 steroid compound found in both animals and few plants mainly in pollen
5-Androstenediol stimulate immune function and enhance survival during bacterial, viral and parasitic infections (Loria et al. 1988; Loria and Padgett, 1992; Gianotti, 1996; Padgett et al. 1997; Suitters et al. 1997; Ben-Nathan et al. 1999; Whitnall et al. 2000). It has also displayed beneficial effects after burn injury, trauma and sepsis (Araneo and Daynes, 1995; Shimizu et al. 2006; Szalay et al. 2006). Further, it stimulates the production and activation of cells of the innate immune system (Whitnall et al. 2000, 2001 and 2002). 5-Androstenediol is being investigated as both a radio protectant (pretreatment) (Whitnall et al. 2002) and a post-irradiation treatment (Stickney et al. 2006 and 2007).

**Compound 2-AD6:** The results of infrared spectroscopy revealed the presence of -OH groups as strong absorbance frequency was observed at 3443 cm\(^{-1}\). A strong absorbance frequency at 2918 cm\(^{-1}\) revealed the presence of alkyl group. The strong absorbance frequency at 1464 cm\(^{-1}\) observed for the C=C stretching. The results of proton nuclear magnetic resonance revealed the presence of five aromatic protons confirmed by the peaks appearing at the region of 7.94-8.3 \(\delta\) value. The peak appeared at the region of 7.9 and 4.9 \(\delta\) value indicated the presence of -OH groups. The two doublets for -CH=CH appeared at the region of 6.2 \(\delta\) values. The peaks for CH\(_2\) protons are appeared at the region of 3.3 and 2.9 \(\delta\) values. The multiplets appeared at the region of 0.8 to 1.4 \(\delta\) value is for -CH\(_3\) protons. The peak observed at Mass peak 379 (positive mode) confirms the molecular weight of the compound and thus it is concluded as cathafuran B.

Cathafuran B (Pubchem ID: CID44139000), is a flavonoid exclusively found in genus Morus. It has been isolated for the first time from the stem bark of Morus cathayana. It is reported to exhibit moderate activities against five human cancer cell lines (Ni et al. 2009).

**Compound 3-AD11:** The results of infrared spectroscopy revealed a strong absorbance frequency at 3362 cm\(^{-1}\) for the -OH groups and strong absorbance frequency at 2924 cm\(^{-1}\) for the alkyl group. For -C=O group, the
peak was observed at 1658 cm$^{-1}$ region. The strong absorbance frequency at 1454 cm$^{-1}$ indicated C=C stretching. The results of proton nuclear magnetic resonance revealed the presence of acid proton as a singlet was appeared at the region of 8.7$\delta$ value. The peak appearing at the region of 5.5$\delta$ value confirms the presence of -OH group. The peaks for CH$_2$ protons were appeared at the region of 2.1-4.0$\delta$ values. The multiplets appeared at the region of 1.1 to 2.0$\delta$ value is for -CH$_3$ protons. The peak observed at Mass peak 457 (positive mode) confirms the molecular weight of the compound as ursolic acid.

Ursolic acid (Pubchem ID: CID220774) is a secondary plant metabolite, usually present in the stem bark, leaves or fruit peel of many plants, such as Mirabilis jalapa, apple (Malus domestica) fruit peel, marjoram (Origanum majorana) leaves, oregano (Origanum vulgare) leaves, rosemary (Rosmarinus officinalis) leaves, sage (Salvia officinalis) leaves, thyme (Thymus vulgaris) leaves, lavender (Lavandula angustifolia) leaves and flowers, eucalyptus (Eucalyptus) leaves and bark, black elder (Sambucus nigra) leaves and bark, hawthorn (Crataegus spp.) leaves and flowers, coffee (Coffea arabica) leaves (Jager et al. 2009; Szakiel et al. 2012). The health promoting activities of this compound have been unknowingly used for centuries, as an ingredient of herb extracts employed in folk medicine. Contemporary scientific research showed that several pharmacological effects, anti-tumor, hepatoprotective, anti-inflammatory, anti-ulcer, antimicrobial, anti-hyperlipidemic and antiviral activities are due to ursolic acid. However, its anti-inflammatory (topical), anti-tumor (skin cancer), and antimicrobial properties are pertinent to the cosmetic industry (Checker et al. 2012; Kim et al. 2013; Ku et al. 2013; Chun et al. 2014; Wozniak et al. 2015).

**Compound 4-AD13:** The results of infrared spectroscopy revealed a strong absorbance frequency for -OH groups at 3332 cm$^{-1}$ and a strong absorbance frequency at 1445 cm$^{-1}$ was observed for the C=C stretching. The results of $^1$HNMR spectra confirmed the presence of 3-OH protons, as a singlet appeared at the region of 4.8 and 5.3$\delta$. The peaks appeared at the region of 7.0-
7.68 value is for aromatic protons. The peak observed at Mass peak 241 (negative mode) of LC-MS confirmed the molecular weight of the compound as moracin M.

Moracin M (Pubchem ID: CID185848) belonging to the class of flavonoids extensively found in Morus genus. Moracin M displayed antimicrobial, scavenging and anti-inflammatory activities (Sharma et al. 2001; Fukai et al. 2003; Sohn et al. 2004; Bellik et al. 2012).

PHARMACOLOGICAL STUDIES
Evaluation of in vitro antioxidant activity

Antioxidant compounds in plants play an important role as a health-protecting factor. There are a number of clinical studies suggesting that the antioxidants in grains, oil seeds, fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller et al. (2000).

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants (Aruoma and Cuppett, 1997).

In the present investigation, various in vitro antioxidant assays have been performed to monitor and correlate the antioxidant activity of different
solvent extracts of *Morus alba*, *Morus serrata* and *Morus laevigata* and the results are discussed below.

Frankel and Koleva suggested that, the use of different methods is necessary in antioxidant activity assessment (Frankel *et al.* 1994; Koleva *et al.* 2002; Kulisic *et al.* 2004). In the present study, the methanolic extracts of *Morus* have exhibited significant antioxidant activities for scavenging, chelating and reducing power when compared to chloroform and petroleum ether extracts. Further, it was proved by quantitative assays *viz.* total phenolic, flavonoid and total antioxidants. Among the three species, *M. alba* was proved to be superior than the other two species. The data therefore suggest that the extracts of *Morus* are a potential source of natural antioxidants. This is due to the presence of phytoconstituents *viz.* alkaloids, sterols, phenolic compounds (flavonoids), glycosides *etc.* which have been known for their antioxidant property (Rauf *et al.* 2014). Many flavonoids and polyphenols can exhibit antioxidant activities as their extensive, conjugated π-electron systems allow ready donation of electrons, or hydrogen atoms from the hydroxyl moieties to free radicals (Bors *et al.* 1987). Most polyphenols especially flavonoids are very effective scavengers of hydroxyl and peroxyl radical (Manach *et al.* 1996). The total antioxidant estimation is not the contribution of any individual antioxidant but to the total antioxidant capacity. Majority of the antioxidant activities are may be from phenolic compounds but other than phenolics, small molecules such as ascorbic acid, R-tocopherol, β-carotene, and reduced glutathione also play a crucial role (Prior *et al.* 1996; Kaur and Kapoor, 2002). Thus, the present study reveals that all the extracts possess potent antioxidant capacity and supports the therapeutic benefits of mulberry leaves which have been used in China for hundreds of years to treat hyperglycemia, inflammation, cough, hypertension, cancer and fever (Bown, 1995; PPRC, 2005).

There are several reports of extraction of antioxidants from plant materials *viz.* *Salvia reflexa* (Malencic *et al.* 2000); *Cetraria islandica* (Gulcin
et al. 2002); Ardisia compressa (Sonia and de Mejia, 2004); Cytisus scoparius (Raja Sundararajan et al. 2006); Zanthoxylum piperitum (Yamazaki et al. 2007); Carya cathayensis (Chenggang Zhu et al. 2008); Boerhaavia diffusa (Rachh et al. 2009); Cassia occidentalis (Mehta et al. 2010); Tecoma stans (Govindappa et al. 2011); Pongamia pinnata (Saiprasanna et al. 2012); Ganoderma lucidum (Huihu et al. 2013); Sonchus oleraceus (Jain and Singh, 2014); Alocasia indica (Swagata et al. 2014); Vaccinium corymbosum L (Contreras et al. 2015); Acacia catechu (Saha et al. 2016).

Correlation analysis, a bivariate analysis was used in the present work for ascertaining the strength of the relationship between the variables. The degree of such relationship can be established by calculating the correlation coefficient (r), which gives a quantitative measure of the degree of closeness between the variables. On the other hand, regression analysis was used for measuring the probable form of the relationship between the variables (Khan and Khanum, 2004). The degree of correlation ranging between 0.7 and 0.9874 suggests a simple, positive and high degree of correlation existing between the variables tested. It is evident from the results that the quantitative estimations performed in the study are positively correlated with the antioxidants studies wherein total antioxidant and total phenolics recorded a high ‘r’ value of 0.9151 and 0.9117 respectively followed by total flavonoid with 0.7989.

Several reports have indicated that there is a direct correlation between antioxidant activity and the presence of phenolics, flavonoids and total antioxidants in the plants. (Sun et al. 2002; Lu et al. 2003; Ye et al. 2005; Mau et al. 2006; Kobus et al. 2009; Mai et al. 2009; Imran et al. 2010). The marginal difference in the averages of correlation coefficient exhibited between total antioxidant and total phenolics might be due to the presence of non-phenolic antioxidants viz. proteins, ascorbate and carotenoids (Kaur and Kapoor, 2002).

The average ‘r’ value was found to be highest in DPPH radical scavenging activity (0.9258) followed by other assays under study irrespective
of quantitative phytochemical estimations, indicating that it is a best suited and reliable radical scavenging activity (Mohadjerani, 2012; Nur-Alam et al. 2013). The linear expression obtained from regression analysis is helpful in measuring the variables in terms of qualitative and quantitative parameters.

It is clear from the study that the tested mulberry species manifested differential expression of antioxidant capacity due to their phytoconstituents. The present study reveals that all the extracts of mulberry possess potent antioxidant capacity and supports the therapeutic benefits of mulberry tea used in some parts of China. Natural antioxidants, particularly in mulberry is rich in phenolic compounds especially flavonoids and anthocyanins and holds free radical scavenging potential (Kim et al. 1999, 2000; Darias-Martin et al. 2003).

**Anticancer activity**

Cancer is a class of diseases characterized by unregulated cell growth. The World Health Organization has reported that approximately 13% of all deaths in the world are caused by cancer each year. Chemotherapy is an essential strategy for the treatment of disseminated cancers; however, its efficiency is restricted by both intrinsic and acquired cell resistance to drugs. To circumvent chemo-resistance to conventional anticancer drugs, anticancer compounds with new cellular targets are needed. This observation stimulates the search for new anticancer agents and in this regard, the investigation of naturally originating compounds could be very valuable.

All through the medical history, nature has been the excellent and reliable source of new drugs, including anticancer agents. Natural sources like plants have always been useful sources of antitumor or cancer prevention compounds (Reddy et al. 2003; Guo et al. 2010). From the currently used anticancer chemotherapeutic drugs, approximately 70% are derived from natural sources (Karikas, 2010). Evidence from recent publications indicates that natural products, especially the secondary metabolites are potential source and give high yield anticancer drugs (Simmons et al. 2005; Blunt et al. 2009). Compounds from natural source are studied extensively with respect to
structural modification in order to explore their further use in pharmacy and medicine in the prevention and treatment of cancer (Kluge and Petter, 2010).

The present study was carried out to evaluate the cytotoxicity, antitumor activity of Morus alba, Morus serrata and Morus laevigata.

The MTT assay is a sensitive, quantitative and reliable assay that measures viability, proliferation and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzyme in living cells to convert the yellow water soluble substrate 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product that is insoluble in water. The amount of formazan produced is the cell number in a range of cell lines (Mosmann, 1983; Gerlier and Thomasset, 1986; Gralier et al. 1988). Tetrazolium dye reduction is dependent on NAD(P)H-dependent oxidoreductase enzymes largely in the cytosolic compartment of the cell. Therefore, reduction of MTT and tetrazolium dyes depends on the cellular metabolic activity due to NAD(P)H flux. Cells with a low metabolism such as thymocytes and splenocytes reduce very little MTT. In contrast, rapidly dividing cells exhibit high rates of MTT reduction. The MTT assay is more useful in the detection of cells that are not dividing but are still active. It can, therefore, be used to distinguish between proliferation and cell activation (Gerlier and Thomasset, 1986). The technique permits the processing of a large number of samples with a high degree of precision using a multi-well scanning spectrophotometer (micro-ELISA reader).

In the present investigation, the in vitro anticancer studies were performed against human cancer cell lines (MCF7 and 3T3), and MTT assay was used to analyze the cell growth inhibition and cell viability. Among the methanolic extracts of three species of Morus the results were observed in a dose dependent manner. The methanolic extract of Morus laevigata showed highest growth inhibition in MCF7 (breast cancer cell lines) with an IC₅₀ value
of 546.08µg/ml when compared to *Morus serrata* (579.16µg/ml) and *Morus alba* (595.33µg/ml). Similar results are observed with respect to 3T3 (non-cancer immortal cell lines), wherein *Morus laevigata*, *Morus serrata* and *Morus alba* registered IC$_{50}$ values of 359.17µg/ml, 386.59µg/ml and 376.66µg/ml respectively.

MTT assay as a reliable tool in anticancer studies has been reported by several researchers (Mosmann *et al.* 1983; Cory *et al.* 1991; Berridge *et al.* 1993 and 2005; Berridge *et al.* 2005; Stockert *et al.* 2012; Shahneh *et al.* 2013; Chan *et al.* 2015) and the results of the present cytotoxicity study is in conformity with these investigations.

Tumor growth and metastasis are dependent on the formation of new blood vessels. The most elegant investigation of the correlation between the onset of angiogenesis and tumor growth was carried out by Folkman and Shing (1992). The clinical usage of herbal medicine could have an impact on therapy for cancer. It is becoming increasingly likely that antineoplastic drugs may play a cytotoxic role in cell proliferation. Ehrlich ascites tumor cells (EAT), very convenient in cancer research, aroused as spontaneous mammary gland carcinomas in senile mice. These cells actively multiply in the peritoneal cavity, so that a large number of cells in suspension are available (Stewart *et al.* 1959). Ehrlich ascites tumor is a rapidly growing carcinoma with very aggressive behavior (Segura 2000). It is able to grow in almost all strains of mice. The Ehrlich ascetic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascetic fluid formation (Fecchio *et al.* 1990). The ascetic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells (Shimizu *et al.* 2004).
The experiments are carried out to evaluate the *in vivo* antitumor activity of *Morus alba*, *Morus serrata* and *Morus laevigata* on EAT bearing mice. The methanol extract treated animals at the different doses of 200; 300 mg/kg inhibited the body weight, tumor volume, packed cell volume and decreased the tumor cell count. Reliable criteria for judging the value of any anticancer agents is the prolongation of life span of animals (Hogland, 1982). A decrease in tumor volume and viable tumor cell count finally reduced the tumor burden and enhanced the life span of EAT bearing mice.

The mean survival time of the EAT cell line bearing mice showed 10.2 days, while it increased to 13.2 and 14.4 days in *Morus alba* methanolic extract treated groups and 12.84 and 13.86 days in *Morus serrata* methanolic extract treated group at 200 and 300mg/kg concentration. *Morus laevigata* however recorded the highest mean survival time of 14.2 and 16.2 at 200 and 300mg/kg concentrations respectively. Further, methanolic extract of *Morus laevigata* at 200 and 300mg/kg concentrations showed remarkable activity in terms of reduced body weight (7.2 and 6.3gm), reduced tumor volume (5.4 and 4.8ml), reduced packed cell volume (2.64 and 2.2ml), reduced viable tumor cell count (44.55 and 37.25) in a dose-dependent manner when compared to that of EAT control group. Among the methanolic extract of *Morus alba* at 200 and 300mg/kg concentrations, the anticancer effects observed in terms of reduced body weight, reduced tumor volume, reduced packed cell volume and reduced viable tumor cell count were 8.75 and 7.4; 8.4 and 7.8; 4.24 and 3.9 and 49.6 and 39.25 in a dose-dependent manner respectively when compared to that of EAT control group. With respect to *Morus serrata* methanolic extract treated group, activities in terms of reduced body weight was found to be 8.81 and 7.2, reduced tumor volume it was 38.56% and 51.18%, reduced packed cell volume it was 4.42 and 3.4 and in terms of reduced viable tumor cell count it was 44.55 and 37.25. It is interesting to note that, among the three species under study,
Morus laevigata was found to be potent for all the parameters evaluated substantiating its anticancer effects.

The presence of biologically active substances such as alkaloids, steroids, triterpenoids and flavonoids in the leaf extracts of three plants under study may be responsible for the antitumor activity as these compounds are proven to exhibit anticancer properties (Douglas, 2000; Matsumoto et al. 2006; Shah et al. 2011; Filip et al. 2013). Further, presence of specific anticancer compounds in mulberry viz. ursolic acid, oxyresveratrol, moracin and deoxynojirimycin-1 (Kumar and Chauhan, 2008) etc. could be contributing to significant antitumor activity.

Anticancer investigation is a matter of great interest worldwide in search of potent anticancer drug and hence incessant efforts have been made by the researchers viz. Hanan et al. (2010) in Ocimum basilicum, Kiranmayi et al. (2011) in Argemone mexicana, Marchetti et al. (2012) in Calea pinnatifida, Henry et al. (2013) in Tillandsia recurvata, Manglani et al. (2014) in Barleria grandiflora, Ghosh et al. (2015) in Dioscorea bulbifera and Cimmino et al. (2016) in Impatiens glandulifera.

**Analgesic activity**

Pain, even though is an unpleasant sensation, is mainly a protective mechanism for the body (Kanodia, 2008). It is a consequence of complex neurochemical processes in the central and peripheral nervous systems. Typically, it is a direct response to an event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause or it can also occur as a consequence of brain or nerve injury. Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively.
The non-opioid analgesics relieve pain without interacting with opioid receptors, reduce elevated body temperature, possess anti-inflammatory property and are non-addicting drugs. These effects are achieved with doses that do not produce significant depression of CNS. The NSAIDs can be classified mainly into two groups, namely, non-selective COX inhibitors (acetyl salicylic acid, paracetamol, phenylbutazone, diclofenac, ibuprofen, piroxicam etc.) and selective COX-2 inhibitors (nimesulide, meloxicam, celecoxib, rofecoxib). Though these drugs have different chemical structures, they produce qualitatively similar actions. During inflammation, pain and fever, arachidonic acid is liberated from phospholipid fraction of the cell membrane. This acid is then converted via cyclo-oxygenase (COX-1 and COX-2) pathways to prostaglandins. These prostaglandins sensitize blood vessels to the effects of inflammatory mediators that increase permeability. The prostaglandins particularly PGE and PGI produce hyperanalgesia associated with inflammation. They sensitize the chemical receptors of the afferent pain endings to other mediators such as bradykinin and histamine. Further, release of prostaglandins in the CNS may lower the threshold of the central pain circuits.

Opioid are drugs which have morphine like action viz. relief of pain and depression of the CNS. The opioid drugs produce their effects by combining with opioid receptors, which are widely distributed in the CNS and other tissues. The opioid receptors have been classified into mu, delta, kappa (K₁ and K₂) and sigma types. The vast majority of opioid drugs used as analgesics are agonists at mu receptors. The major drawbacks of these opioid analgesics are the development of tolerance and physical as well as psychological dependence.

Analgesic effects of different solvent extracts of three species of mulberry are performed at different concentrations (ie. 200 and 400 mg/kg) by tail immersion method. The data recorded after one hour showed that, the control group registered reaction time of 2.43 and standard 9.17 whereas, the
different plant extracts of the three species showed variations in the analgesic effects in a dose dependent manner. Animals treated with a dose of 200mg/kg, significant increase in reaction time was shown by all the groups except the petroleum ether extracts of the three plants. Animals treated with 400mg/kg dosage, all the extracts showed significant analgesic activity in terms of reaction time except petroleum ether extract of *Morus serrata* and *Morus laevigata*. It was 5.15±0.13; 8.23±0.15; 10.58±0.16 in petroleum ether, chloroform and methanol extracts of *Morus alba* respectively. The chloroform and methanol extracts of *Morus serrata* recorded 7.52±0.16 and 9.92±0.27 respectively and *Morus laevigata* recorded a reaction time of 6.93±0.13 and 9.37±0.15 for chloroform and methanol extracts respectively (P<0.01). It is evident from the data that, amongst the different solvents used; highest analgesic activity was shown by methanol extract followed by chloroform and petroleum ether. Further, among the three species under study, it is notable that *Morus alba* was found to be superior followed by *Morus serrata* and *Morus laevigata* in terms of pain relieving abilities.

Pain is a complex event, centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti, 1983; Headley, 1985; Wigdor, 1987; Pasero, 1999). Preliminary qualitative phytochemical screening reveals the presence of alkaloids, flavonoids, glycosides, terpenoids, tannins etc. in *Morus species*. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rao *et al.* 1998; Rajnarayana *et al.* 2001). There are also reports on the role of tannins in anti-nociceptive activity (Vanu *et al.* 2006), besides alkaloids are well known for their ability to inhibit pain perception (Uche *et al.* 2008).

The present work corroborates the investigations carried out in several medicinal plants *viz.* *Caesalpinia ferrea* (Carvalho *et al.* 1996); *Psidium guajava* (Kulkarni *et al.* 1999); *Piperomia pellucid* (Peter *et al.* 2001);
Carthamus lanatus (Bocheva et al. 2003); Euphorbia decipiens (Ahmad et al. 2005); Commiphora caudata (Mohan et al. 2009); Trichosanthes bracteata (Verma et al. 2010); Terminalia arjuna (Biswas et al. 2011); Camellia oleifera (Yong et al. 2012); Vetiveria zezanioides (Kamble et al. 2013); Sarcochlamys pulcherrima (Ibrahim et al. 2014); Grewia crenata (Ukwuani et al. 2014); Ocimum suave (Tesema et al. 2015); Cistus ladanifer L (Youbi et al. 2016) for analgesic activities.

**Anti - inflammatory activity**

Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan induced oedema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, all of which also cause pain and fever (Asongalem et al. 2004). Inhibitions of these mediators from reaching the injured site or from bringing out their pharmacological effects normally ameliorate the inflammation and other symptoms. This study has shown that the methanolic leaf extracts of Morus species possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation (Ozaki 1990; Silva et al. 2005). Carrageenan oedema is a multimediated phenomenon that liberates diversity of mediators. It is believed to be biphasic; the first phase (1h) involves the release of serotonin and histamine while the second phase (over 1h) is mediated by prostaglandins, the cyclooxygenase products, and the continuity between the two phases is provided by kinins (Silva et al. 2005; Perianayamgam et al. 2006).

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert, 1994). The time course of edema development in carrageenan-induced paw edema model in rats
is generally represented by a biphasic curve (Vinegar, 1969). The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The second phase is sustained by prostaglandins released and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages component (Crunkhorn, 1971; Brito, 1988) and play a major role in the development of the second phase of inflammatory reaction.

In the present study, acute inflammatory condition is produced in the animals by carrageenan induced pedal inflammation and the results reveals that administration of carrageenan to the rats showed a rise in paw volume at different time intervals in control group whereas, it was reduced in the indomethacin treated group. Even though plant extracts treated groups exhibited differential anti-inflammatory activity, significant (P<0.01) reduction in paw volume was recorded at 400mg/kg by *Morus alba* and *Morus serrata* at the third and fourth hour interval in all the extracts. However, in *Morus laevigata*, significant (P<0.01) anti-inflammatory effect was noted in forth hour in chloroform and at third and fourth hour intervals in methanol extract treated group. It is clear from the study that, the anti-inflammatory effect was more pronounced at later phases of time interval viz. third and fourth hour and was proved to be highest in methanolic extracts followed by chloroform and petroleum ether. Further, the order of efficiency in terms of anti-inflammatory effect were *Morus alba* > *Morus serrata* > *Morus laevigata*. It can be inferred that the inhibitory effect of the extracts on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. The phytochemical screening shows the presence of flavonoids in all the extracts of the three plants and is known to inhibit prostaglandin synthetase (Ramaswamy, 1985). Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (Mossai et al. 1995, Sawadogo et al. 2006; Sheeja et al. 2006; Wantana, 2009; Gulnaz et al. 2014; Safkath et
al. 2014), the results of this study are an indication that *Morus alba* can be effective in acute inflammatory disorders compared to *Morus serrata* and *Morus laevigata*.

The present work is in agreement with the investigations carried out in several medicinal plants *viz*, *Curcuma amada* (Mujumdar *et al.* 2000); *Securidaca vitex negundo* (Rasadah *et al.* 2005); *Ruta graveolens* (Ratheesh and Helen, 2007) and *Aloe buettneri* (Metowogo, 2008); *Bambusa vulgaris* (Carey *et al.* 2009); *Rubia cordifolia* Linn (Tailor *et al.* 2010); *Barleria prionitis* L (Khadse *et al.* 2011); *Murraya koenigii* (Darvekar *et al.* 2011), *Ajuga bracteosa* Wall (Singh *et al.* 2012); *Tecomastans* (Prasanna *et al.* 2013); *Garcinia pedunculata* (Mundugaru *et al.* 2014); *Senecio flammeus* (Xiao *et al.* 2014); *Achyranthes aspera* Linn. (Ndhlala *et al.* 2015); *Artemisia maritima* L. (Irum *et al.* 2015); *Bauhinia pulchella* (Lopes *et al.* 2016).

**Wound healing activity**

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions (Senthil Kumar *et al.* 2006). Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin (Meenakshi *et al.* 2006; Ghosh *et al.* 2012). In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion (Enoch and John Leaper, 2005). Wound healing is a complex cellular event by which a damaged tissue restored as closely as possible to its normal stage. The healing process depends upon the reparative abilities of the tissue, the type and extent of damage and general state of health of the tissue. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase and finally
the remodeling phase which ultimately determines the strength and appearance of the healed tissue (Sumitra et al. 2005).

Wound healing process holds several steps which involve coagulation, inflammation, formation of granulation tissue, matrix formation, remodeling of connective tissue, collagenization and aquisition of wound strength (Suresh Reddy et al. 2002; Nayak et al. 2006). Research on wound healing agents is one of the developing areas in modern biomedical sciences and many traditional practitioners across the world particularly in countries like India and China have valuable information of many lesser-known hitherto unknown wild plants for treating wounds and burns (Kumar et al. 2007). Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of wounds related disorders (Krishnan, 2006; Fahimi et al. 2015).

In the present investigation, wound healing abilities are evaluated by adopting two wound models viz. excision and incision. The results of the excision wound revealed that, even though significant wound closure was noted on 8th day in only chloroform (44.31±2.12) and methanol (46.14±1.16) extracts of Morus alba (P<0.01) and on 12th day by chloroform and methanol extracts (64.21±1.06, 76.05±1.84) and methanol extract of Morus serrata (66.17±1.46), all the treated groups of Morus alba have demonstrated significant wound contraction on 16th day (85.12±1.52, 88.18±2.21 and 92.06±1.62) while chloroform and methanol extract of Morus serrata (85.13±1.18, 88.21±1.32) and methanol extract of Morus laevigata (86.50±1.57) have exhibited wound healing effects (P<0.01).

As far as mean time required for complete epithelialization, the control group animals took a mean time of 23.11 days whereas in standard group it was found to be 16.36 days. Only methanol extracts of the three species recorded a significant reduction in mean time of 17.52, 18.18 and 18.53 days respectively when compared to untreated groups for complete epithelialization.
The breaking strength of the skin in incision wounds was increased in the *M. alba* extract treated groups (methanol, chloroform and petroleum ether) to a significant extent, *i.e.* 1498.88, 926.52 and 628.18 while in *M. serrata* and *M. laevigata* extract treated groups it was 1312.55, 686.78 and 1258.33, 876.67 in methanol and chloroform extracts respectively. The results were comparable to the standard drug, nitrofurazone. It is evident from the results that, the *Morus alba* was potent in terms of wound healing followed by *Morus serrata* and *Morus laevigata*. Further, methanol solvent was proved to be the ideal solvent to achieve higher wound healing efficiency.

*Morus* is a very good source of ascorbic acid and also contains carotene, Vitamin B1, folic acid, folinic acid, isoquercetin, quercetin, tannins, flavonoids and saponins, which act as a good source of natural antioxidants. Flavonoids have been documented to possess potent antimicrobial, antioxidant and free radical scavenging effect, which is believed to be one of the most important components of wound healing (Devi and Shyamala, 1999; Nikkhah *et al.* 2008). There are reports which indicated that the plants having antioxidant property would also enhance wound-healing activity (Shirwaikar *et al.*, 2003). The other phytoconstituents present in *Morus* species *viz.* quercetin 3-(malonylglucoside), rutin, isoquercitin, cyaniding, rutinoside and cyanidin 3-glucoside which either due to their individual or additive effect fastens the process of healing (Annan *et al.* 2008; Kaushik *et al.* 2013) and therefore could be the reason for wound healing activity. The present study documents the wound healing property of *Morus* and validates the folk medicinal claim.


**CNS Depressant activity**

CNS depressants reduce the locomotor activity in experimental animals. Locomotor activity is considered as an index of alertness, and a decrease indicates a sedative effect (Thakur and Mengi, 2005). The standard drug Chlorpromazine is being used in major psychosis like schizophrenia and to reduce the destructive behavior in children. The reports also indicated the use of this drug in experimental animals to evaluate the CNS depressant effect of the plant based drug (Okugawa et al. 1996).

The locomotor activities of different solvent extracts of three species of mulberry are performed at different concentrations i.e. 200 and 400mg/kg. The data showed after one hour of observation that, the different plant extracts of the three species showed variations in the locomotor effects in a dose dependant manner. The results revealed a significant reduction in locomotor activity (P<0.01) of 171.94, 212.32 and 183.96 by the animals treated with petroleum ether, chloroform and methanol extracts of Morus alba while it was 181, 230.8 and 207 in Morus serrata and 201.88, 257.71 and 211.81 in Morus laevigata respectively at 400mg/kg concentration. Even though the reduction in locomotor activity was observed at 200mg/kg dosage the data are statistically not significant indicating dosage efficiency. Further, perusal of the results revealed that the extracts of Morus alba were more effective in CNS depression than Morus serrata and Morus laevigata in all the three extracts and the depression was more pronounced in petroleum ether extracts of the three species.

The effect of these extracts may probably be due to the increase in the concentration of GABA in the brain. Gamma animobutaric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different
anxiolytic, muscle relaxant, sedative-hypnotic drugs elucidate their action through GABA (Angad Verma et al. 2010), therefore it is possible that extracts of Morus alba, Morus serrata and Morus laevigata leaves may act by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization leading to a decrease in the firing rate of critical neurons in the brain or may activity through direct activation of GABA receptor by the extracts. The variation in the potency of the extracts may be due to the difference in the concentration of the constituents (Nyeem et al. 2006; Ali et al. 2014; Emran et al. 2014).

Several investigations have revealed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders (Bhattacharya and Satyan, 1997; Kolawole et al. 2007; Verma et al. 2010; Ripa et al. 2014). Since the phytochemical investigations in the three plants tested showed the presence of similar constituents and therefore could be attributed for their CNS depressant activity. Very little work has been carried out to investigate the CNS activity in Morus (Adhikrao et al. 2008; Yadav et al. 2008). The results suggest that the leaves of Morus may have potential clinical application in the management of psychiatric disorders.

The CNS activity has also been carried out in several medicinal plants viz. Nyctanthes arbor-tristis (Das et al. 2008); Sterculia guttata (Katade et al. 2009); Momordica dioica Roxb (Maharudra et al. 2010); Acalypa indica (Ramakrishnan et al. 2011); Cocos nucifera (Dilipkumar et al. 2011); Derris trifoliate (Mamoon et al. 2012); Kalanchoe pinnata Lam (Matthew et al. 2013); Erythrina variegate (Murugalakshmi et al. 2014); Typha angustata (Ashok et al. 2014); Alpinia oxyphylla (Chauhan and Swapna, 2015).

Anthelmintic activity

Helminthiasis is one of the major problems of livestock production throughout the world, particularly in tropical and subtropical areas. The World Health Assembly, in a number of resolutions has emphasized the need to the use of natural products with therapeutically proven efficacy particularly in
patients residing in tribal areas who are very much prone to the attack of several infections due to lack of knowledge about proper sanitation and can affect most populations with major economic and social consequences (Ghosh et al. 2005). Further, helminthes also affect millions of livestock resulting in considerable economic losses in domestic and farm yard animals because of limited availability and affordability of modern medicines. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance (Mehjabeen et al. 2011).

The helminthes which infect the intestine are cestodes eg. tape worm (Taenia solium), nematodes eg. hookworm (Ancylostoma duodenale), round worm (Ascaris lumbricoids) and trematodes or flukes (Schistosoma manso1 and S. hematobolium). The disease originated from parasitic infections causing severe morbidity includes lymphatic filariasis, onchocerciasis and schistosomiasis. Ideally an anthelmintic agent should have broad spectrum of action, high percentage of cure with a single therapeutic dose, free from toxicity to the host and should be cost effective. None of the synthetic drug available meets this requirement. Even most common drugs like piperazine salts have been shown to have side effects like nausea, intestinal disturbances and giddiness (Liu, 1996), resistance of the parasites to existing drugs (Walter, 1985) and their high cost warrants the search for newer anthelmintic molecules. Therefore, many investigators are focusing researches on the alternatives to the chemical control of helminthes particularly herbal drugs. There are various plants which are reported as anthelmintic (Patil et al. 2009). Considerable research in this regard has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For example, plants that contain condensed tannins, a class of phenolic secondary metabolites, have these effects (Jalalpure et al. 2003). Search for anthelmintic factor in plants therefore remains a potential area of investigations.
Most of the screening reported are in vitro studies using some worm samples like *Pheretima posthuma, Ascaris galli, A. lumbricoides* etc. Adult Indian earth worm, *P. posthoma* has been used as test worm in most of the anthelmintic screening, as it shows anatomical resemblance with the intestinal roundworm parasite of human beings (Vidyarthi, 1967; Chatterjee, 1967; Vigar, 1984; Thorn, 1977). Because of easy availability, *P. posthoma* earthworms are used as suitable models for screening of anthelmintic drug. These in vitro screenings are important as they give basis for further in vivo studies (Ravindra, 2008).

The present study revealed that the sequential extracts of *Morus alba*, *Morus serrata* and *Morus laevigata* possess potent anthelmintic property in a dose dependent manner for the parameters studied viz. paralysis and death which is quite comparable with standard anthelmintic drug in the organism tested. It is due to the presence of active principles in the plant extracts. It acts as potent anthelmintic, because the extracts of the plant contains flavonoids, triterpenoids, alkaloids, steroids and tannins. Specifically, tannins and flavonoids present in the extracts may be attributed to the pronounced anthelmintic activity (Waller, 1997; Athnasiadou *et al.* 2001). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite and cause death (Thompson and Geary, 1995).

The major effect of anthelmintic compounds could be due to decrease in motility, paralytic action, damage to the mucopolysaccharide membrane and on the neuromusculature of helminthes worms. The metabolic pathways in general and carbohydrate pathways in particular and neuromuscular co-ordination are the major targets (Dhar, 1965). Specifically, tannins and alkaloids present in the mulberry extracts may be attributed to profound anthelmintic activity (Atnasiadou *et al.* 2001; Dash, *et al.* 2002; Mali *et al.* 2007; Deore and
Khadabade, 2010; Dhembare and Kakad, 2015). The anthelmintic activity of the various extracts validates the folk claim of *Morus*.

*In vitro* anthelmintic activity is a matter of several investigations in various plants and the anthelmintic activity of mulberry is in conformity to these reports *viz.* *Flemingia vestita* (Tandon and Das, 2007); *Carthamus tinctorious* (Paramesha *et al.* 2009); *Chlorophytum borivilianum* (Deore and Khadabade, 2010); *Tamarindus indica* (Das *et al.* 2011); *Cassia auriculata* L (Gaikwad *et al.* 2011); *Luffa cylindrica* (Partap *et al.* 2012); *Hibiscus* and *Rosa sinensis* (Pekamwar *et al.* 2013); *Cassia auriculata* (Sachin Chaudhary and Amit Kumar, 2014); *Cassia occidentalis* (Sayyad *et al.* 2014); *Artemisia vestita* and *Artemisia maritima* (Irum *et al.* 2015) etc.

**Antimicrobial activity**

Many plants have been used because of their antimicrobial traits and have been investigated by a number of researchers worldwide (Ncube, 2008). The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment (Iwu, 1999). Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka *et al.* 2006) especially in the light of the emergence of drug-resistant microorganisms and the need to produce more effective antimicrobial agents. Bacteria have evolved numerous defenses against antimicrobial agents, and drug-resistant pathogens are on the rise. This resistance is conferred by multidrug resistance pumps (MDRs), membrane translocases that extrude structurally unrelated toxins from the cell. These protect microbial cells from both synthetic and natural antimicrobials (Stermitz *et al.* 2000). Secondary metabolites resemble endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to their recognition in potential target sites (Parekh *et al.* 2005). The use of plant extracts and phytochemicals can be of great
significance in therapeutic treatments and could help curb the problem of these multi-drug resistant organisms. Moreover, the synergistic effects of extracts with antimicrobial activity in association with antibiotics can provide effective therapy against drug resistant bacteria.

Over the past several years, intensive efforts have been made to discover clinically useful antimicrobial drugs, which have been reviewed by many researchers. (Rasadah and Houghton, 1998; Blondeau, 1999; Cowan, 1999; Jacoby, 1999). This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor et al. 2001).

The antimicrobial susceptibility test (AST) is an essential technique in many disciplines of science. It is used in pathology to determine resistance of microbial strains to antimicrobials, and in ethnopharmacology research, it is used to determine the efficacy of novel antimicrobials against microorganisms, essentially those of medical importance. The test is the first step towards new anti-infective drug development. There are various AST methods that are employed by researchers and these could lead to variations in results obtained (Lampinen, 2005).

Despite acknowledged exceptions with certain drug–bacteria combinations, antibacterial drugs are usually divided into two groups: those that are primarily bacteriostatic (ie. that inhibit growth of the organism) and those that are primarily bactericidal (ie. that kill the organism). The in vitro antimicrobial activity of drugs is usually assessed by determining of the MIC and MBC. The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by sub-culturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥99.9%. The MBC is complementary to the MIC; whereas the MIC test demonstrates the
lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. The MIC is a measure of the potency of an antimicrobial drug. Isolates of a particular species will have varying MICs; sensitive strains will have relatively low MICs, and resistant strains will have relatively high MICs. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC.

The results of the antibacterial activity revealed that all the extracts showed noticeable effects in dose dependent manner against the organisms studied at concentrations of 20, 40, 60 and 80mg/ml. Considering the overall mean values of the zone of inhibition, *Salmonella typhi* was found to be highly sensitive followed by *Bacillus subtilis*, whereas *Shigella flexneri* appears to be highly resistant followed by *Proteus vulgaris*. It is also very clear from the data that, the methanolic extracts and *Morus alba* irrespective of the species and solvent extracts exhibited better antibacterial activities respectively. The overall mean values of the zone of inhibition with respect to antifungal activity suggest that, *Candida albicans* is highly sensitive to all the extracts than *Aspergillus niger*.

The MIC of isolated compounds assessed by tube dilution method indicated highest bacteriostatic effect by ursolic acid at 100ug/ml concentration against *Staphylococcus aureus* and *Pseudomonas aeruginosa* while cathafuran B against the latter only whereas, MIC was found to be 200ug/ml for other compounds against the tested organisms. The minimum bactericidal concentration of the isolated compounds against *Staphylococcus aureus* was found to be 200ug/ml for ursolic acid and 400ug/ml for 5-androstenediol, cathafuran B and moracin M. At 200ug/ml concentration cathafuran B and ursolic acid showed minimum bactericidal effect against *Pseudomonas aeruginosa* while it was 400ug/ml for 5-androstenediol and moracin M.

The demonstration of antibacterial activity against bacteria and antifungal activity may be indicative of the presence of broad spectrum
antibiotic compounds (Srinivasan et al. 2001). The activities could be attributed to the presence of flavonoids, triterpenoids, alkaloids, steroids, phenolic compounds and tannins which have multiple biological effects including toxicity to the microorganisms. Flavonoids, phenolic compounds in particular are important for the plant growth and defense against infection and injury. These compounds while exhibiting antioxidant property are usually also act as good antimicrobial agents (Rasadha et al. 1998; Cowan, 1999; Jacoby, 1999; Kahkonen et al. 1999; Cos et al. 2001). The underlying mechanisms could be enzyme inhibition by oxidation (McGaw et al. 2002). Further, the variation in antimicrobial sensitivity may be due to the differences in the chemical nature of the cell wall and cell membrane of each microorganism (Bal-Tembe et al. 1996). Thus the present antimicrobial activity effects of different Morus species might be because of these constituents in addition to the reported phytochemicals, like leachianone, kuwanon-G and 1-deoxynojirimycin (DNJ).

Many plant species has been established to exhibit antimicrobial activities viz. Bacopa monnieri (Ghosh et al. 2006), Euphorbia tirucalli (Asha et al. 2009); Cyclea peltata. (Abraham et al. 2010); Pomegranate rind (Yehia et al. 2011); Cannabis sativa (Ali et al. 2012); Schinus lentiscifolius (Gehrke et al. 2013); Careya arborea (Prabhakaran et al. 2014); Artemisia annua (Tajehmiri et al. 2014); Momordica charantia (Filho et al. 2015); Nigella sativa (Emeka et al. 2015) etc.

**IN SILICO STUDIES ON ISOLATED COMPOUNDS**

The process of drug development aims towards the identification of compounds with pharmacological interest to assist in the treatment of diseases and ultimately to improve the quality of life. The compounds used in pharmacology are mainly small organic molecules (ligands) which interact with specific bio-molecules (receptors/targets). Usually, compounds with some shared physico-chemical property or obtained according to a particular protocol are compiled into large collections that are termed as libraries. Experimental
identification of small molecules with the desired activity can be achieved by a High-Throughput Screening (HTS) (Bielska et al. 2011).

Traditional method of drug discovery involves series of several steps and needs to be carried out sequentially (Augen, 2002). If one of the steps gets slow, it slows down the entire process. High-throughput drug screening (HTS) is time consuming, expensive and very risky process. In contrast, the robust method of High-Throughput Virtual Screening (HTVS) provides the base for integrated hit and lead optimization strategies (Rajesh et al. 2013).

Computer-Aided Drug Design (CADD) is a specialized discipline that uses computational methods for the simulation of drug-receptor interactions. CADD methods majorly involve bioinformatics tools, application and support from information technology, information management, software applications, databases and computational resources for its infrastructure establishment (Pranita et al. 2012). Virtual screening, sometime called in silico screening, is a new branch of medicinal chemistry that represents a fast and cost effective tool for computationally screening databases in search for the novel drug lead. The routes for the virtual screening go back to the structure-based drug design and molecular modeling. Virtual screening uses computer based methods, discover of new ligands on the bases of biological structure (Shoichet, 2004). The basic goal of the virtual screening is the reduction of the enormous virtual chemical space of small organic molecules, to synthesize and/or screen against a specific target protein, to a manageable number of the compound that inhibit a highest chance leading to a drug candidate (Armstrong, 1999; Tondi et al. 1999; Mugge et al. 2003; Hann et al. 2004; Shoichet, 2004; Fara et al. 2006; Fernandes et al. 2009; Clark et al. 2010; Martis et al. 2011).

Many in silico tools can be used to design libraries (Villoutreix et al. 2007) of compounds with drug-like properties. These are predominantly biophysical properties based on empirical rules. A well-known example is Lipinski's "Rule of Five" (Lipinski, 2000) which states that a compound is likely to be "non-drug-like" if it has more than five hydrogen bond donors,
more than 10 hydrogen bond acceptors, molecular mass is greater than 500 and lipophilicity is above 5.

Rule of 5 was employed for the screening of the drug molecules and the results revealed that all the drug candidates satisfied the rule of five except cathafuran B (6.8) and ursolic acid (6.7) where violation is found only in terms of ClogP. The results of drug likeness prediction revealed that the compound moracin M has shown the highest drug likeness score of 1.24 when compared to 5-androstenediol (0.40), cathafuran B (0.15) and ursolic acid (0.65). Further, results of in silico ADME-toxicity studies revealed that, all the four compounds were found to be non-toxic, non-carcinogenic and very soluble in aqueous solvent.

Automated docking using Lamarckian genetic algorithm, implemented in the program AutoDock 4.2, was used to assess the orientation of the ligand molecules bound in the binding site of different target molecules. Among the four compounds tested, 5-androstenediol showed efficient binding in terms of binding energy with glucosamine-6-phosphate synthase (-9.01), cathafuran B with Cox II (-11.71), and GABA receptor (-11.96), ursolic acid with P38 MAP Kinase (-9.97), GSK 3-β (-10.96) and p450 14-alpha-sterol demethylase (-11.12) and moracin M with β- tubulin (-10.09 kJ/mol).

CONCLUSION

The present research programme generated interesting results in terms of health benefits of mulberry by documenting biological activity for multifarious pharmacological parameters tested viz. anticancer, anthelmentic, anti-inflammatory, wound healing, CNS depressant and analgesic properties in addition to antioxidant and antimicrobial activities and establishes the fact that, the mulberry species possess important secondary metabolites which has contributed to the manifestation of their bioactivity and validates the folk claims of therapeutic benefits of Mulberry. The study lead to the isolation of four bioactive compounds viz. 5-Androstenediol, Cathafuran B, Ursolic acid and Moracin M. The isolated compounds were analyzed for in silico toxicological and pharmacological studies in connection with the identification of lead molecule. The study also established that these therapeutic benefits are variable with respect to species.

Even though Morus alba was found to be superior for most of the pharmacological activities studied, it is interesting to note that Morus laevigata was proved to be ideal material in terms of anticancer effects which therefore warrants detailed in vitro and in vivo studies to identify specific bioactive molecule/s and thereupon deduce mechanism based validation. The study also supports several of the health benefits of Morus and extends it to two different nearly unexplored species viz. Morus serrata and Morus laevigata. Since the work is carried out with a comparative outlook could therefore form the basis for the identification and development of highly health-supporting mulberry species and varieties. These results, demonstrating superior phytochemical traits of mulberries, may also provide a basis for planning breeding strategies as well as selecting cultivars with high phytochemical profiles and antioxidant capacities as functional foods for consumers. The present study acts as a prelude for efforts in exploitation of health benefits features of mulberry in addition to its role as a forage/feed in sericulture industry. Any experiments targeting a single pharmacological parameter utilizing the appropriate compound will be highly rewarding.