7.0 DISCUSSION

Indian traditional system of medicine is based on pragmatic facts of the observations and the experience over millennia. Traditional medicine, being a significant element in the cultural patrimony, still remains the main choice for a large majority of people for treating various diseases. The WHO has estimated that 80% of the population of the developing countries depends
on traditional medicine mostly derived from plants for their primary health care needs. The demand of medicinal plants is increasing throughout the world. 90% of the drugs used in Indian systems of medicine and Homeopathy are plants based and collected from wild source. In recent years traditional drugs are receiving great attention all over the world; therefore a great emphasis has been laid to revive the heritage knowledge on the medicinal plants. Medicinal plants have been man’s oldest friends in his efforts at health and healing. Hence the present study is focused to evaluate all the traditional information gathered through various literatures has been taken into our account to valuate in scientific manner.

**Macro and Microscopic studies of bark:**

The macro and microscopical characters of the bark of *Crataeva religiosa* can be used for distinguishing the species even when they are fragmented. *Kokate et al., (2004)* reported that the secondary external tissues lying outside the cambium in stem of root of dicotyledonous plants are known as the bark and consists of three layers viz, cork (phellem); cork-cambium (phellogen) and secondary cortex (phelloderm). The presence of crystals of calcium oxalate within the bark tissues and non granular trichomes are pointers to medicinal lodgments of bioactive principles of the plants. Some useful diagnostic features for the species are the shape and arrangement of starch grains, stone cells, shapes of calcium oxalate crystals and multiseriate medullary rays for the clear identification of this species. The results of these investigations could serve as a basis for proper identification, collection and investigation of the plant.
Physico-Chemical Analysis:

Moisture content:

In the present study the bark sample contains 3.8% moisture. Moisture content (loss of drying) determines the water drying of the drug. Drug containing excess moisture will lead to the activation of enzymes and gives suitable condition for the proliferation of living micro-organisms. Higher water content indicates the presence of larger amount of mucilage or starch and paves way for more chances of microbial degradation and if the value is not too high, it indicates less chance of microbial degradation (Trease and Evans, 1983) Loss on drying is the loss of mass expressed as percent w/w and results are tabulated in Table -1.

Ash content:

The presence of ash in raw material is determined as total ash, acid insoluble and water soluble ash and sulphated ash. The determination of ash value is useful for detecting exhausted drugs and excess of sandy and earthy matter. The total ash usually consists of carbonates, phosphates and silicates of silica. Ash value determination is a good index of quality and is also helpful to some extent in the detection of adulteration. An increase in the ash value when compared to the standardized value is indicative of contamination or adulteration (Trease and Evans, 1983).

Ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drugs results in an ash residue consisting of an inorganic material (metallic salts and silica). This value varies within fairly wide limits and is therefore an important parameter for the purpose of evaluation of crude drugs (Mukharjee, 2005). Therefore percentage of the total ash, sulphated ash, acid insoluble ash and water soluble ash were carried out. The extraction of any crude drug with a particular solvent yields a solution
containing different phyto-constituents. Extractive value is also useful for evaluation of crude drug, which gives an idea about the nature of the chemical constituents present in a crude drug and is useful for the estimation of specific constituents, soluble in that particular solvent used for extraction (Khandelwal, 1998).

In the present study, the total ash content of the sample was 12.45%. Acid insoluble ash value can be used to determine the silica impurities admixed with the drug while collection and harvesting during rain. Water soluble ash value helps in determining the added mineral matter and helps in the interpolation of analysis of powdered drug for their quality. It is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation (Pruthi, 1980).

Total ash value determined was higher in the mature inner bark than apical and middle bark and increased during summer. Among all the bark samples during both the season, highest amount was noticed in mature inner bark (20.0%) and minimum ash content was estimated for apical stem bark (14.6%). These reports are higher than ash values for Baccaurea sumatrana (5.34%) and Pomelia tomentosa (1.15%) as reported by Whitten and Whitten, (1987).

The total ash value, water soluble ash value and acid insoluble ash value of Crataeva tapia leaf is 12.35 %, 0.91% and 5.45% respectively. Since the ash value is constant for the given drugs, this value is one of the diagnostic parameter of the drug. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The aqueous extractive value was found to be higher (27.80%) than the other solvents used viz. benzene, petroleum ether, ethanol and methanol, revealing presence of large amount of water soluble constituents in
the leaves. By conventional procedures, loss on drying was performed showing 71.60% loss on drying \textit{(Patil et al., 2010)}.

**Extractive values:**

The determination of extractable matter refers to the amount of constituents in a given amount of raw material extracted with suitable solvents. Such extractive values provide an indication of the extraction of the polar and non-polar components present in the material. These values have significance in the evaluation of drugs \textit{(Miller, 1973)}.

Extractive value profiles help in the detection of adulterants during the process of authentication of crude and raw drug materials. Earlier work had revealed the extractive value profiles in several other medicinal plants \textit{(Khatoon et al., 2006)}.

**Preliminary phytochemical screening:**

The medicinal plants have some chemical substances called phytochemicals that produce a physiological action on the human body. Phytochemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of particular primary and secondary metabolities in the plant extract of clinical significances.

The phytochemical analysis of diethyl ether, Chloroform, Acetone extracts of leaf, stem and root of \textit{Cleome gynandra L} was analyzed for the compounds such as Tannin, Saponin, Flavonoids, Steroid, Terpenoids, Cardiac Glycosides, Alkaloids, and Anthraquinones. Acetone, Chloroform, diethyl ether of leaf, stem, root were used for phytochemical studies indicated that the leaf, stem and root contain a broad spectrum of secondary metabolites. Tannin, steroids, cardiac glycosides and alkaloids. Likewise Tannin, saponins, steroids, cardiac glycosides were predominantly found in all the tested solvent in plant extracts. Flavanoids were not found in any of the solvent extracts of leaf, stem, and root.
The ethanolic extract of *Cleome gynandra* leaf might exert anti-inflammatory activity by modifying the lysosomal membrane in such a way that it is capable of fusing with the plasma membrane and thereby preventing the release of lysosomal enzymes and could retard complications and spread of the inflammatory process by reducing the destruction of TNF –α during rheumatism.

The traditional use of *Cleome gynandra* L for human ailments have partly explained its use in herbal medicine as rich source of phytochemicals with Tannin, Saponin, Flavonoids, Steroid, Terpenoids, Cardiac glycosides, Alkaloids, Anthrax quinines (Annadurai and Ahmad John, 2014).

In the present study, chloroform and ethanolic bark extracts shows positive response to alkaloids, glycosides, tannins and petroleum ether shows positive response to sterols. The aqueous extract shows negative response to alkaloids, glycosides, sterols, flavonoids, and triterpenoids. The positive response to the above mentioned compounds to the chloroform and ethanolic extracts may be due to the dissolution capacity of phytochemicals in the organic solvents. Flavonoids is comparatively less in all these extracts Which was previously supported by Patil and Gaikwad, (2011). The preliminary data collected is helpful in the preparation of an authentic preliminary phytochemical profile.

The amount of water soluble tannins ranges from 6.78±0.056%. These values are quite lower than *Acacia mangium* (15%-25%) reported earlier by Hoong et al., (2009) and *Stryphnodendron obovatum* (20%) reported by Sanches et al., (2005).

The Quantitative estimation of Tannins and Saponins was carried out according to the method of Anchana Chanwitheesuk et al., (2005) and Obadoni and Ochuko, (2001). It shows the quantitative variations of phytoconstituents in the respective extracts and it directly
reflects its pharmacological potential. The tannins and saponins are potentially anti-inflammatory and analgesic agents and it was reported by Darshan and Doreswamy, (2004) and Safayhi and Saiter, (1997).

**Pharmacological study:**

The inflammatory process may be defined as a sequence of events that occurs in response to noxious stimuli, infection or trauma (Calixto et al., 2004). The classic signs of inflammation are local redness, swelling, pain, heat and loss of function. The events of inflammation that underline these manifestations are induced and regulated by a large number of chemical mediators, including kinins, eicosanoids, complement proteins, histamine and monokines (Percival, 1999).

Due to the increase frequency of NSAID and their common side effects the use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists worldwide (Sofowora, 1982). *Crataeva religiosa* is used as an anti-inflammatory agent in folklore. To give a scientific validation to this plant an attempt was made to study the anti-inflammatory activity.

The various extracts of bark of *Crataeva religiosa* was tested for anti-inflammatory activity against carrageenan induce paw-edema in rats. All the extracts are having anti-inflammatory activity against the carrageenan induced paw oedema in rats. The reductions of paw oedema of rats are compared with the standard drug i.e. indomethacin. The ethanolic and aqueous extract shows significant activity as compared with standard drug indomethacin which was shown in the Table-6.

Sub planter injection of carrageenan in rats shows time dependent increase in paw thickness. Carrageenan induced rat hind paw edema has been widely used for the discovery and
evaluation of many anti-inflammatory agents, since the relative potency estimates obtained from most drugs tend to reflect clinical experience (Winter et al., 1962). Development of edema in the paw of the rat after injection of Carrageenan is a biphasic event (Vinegar et al., 1969). The first phase is due to the release of histamine and serotonin and the second phase is due to the release of prostaglandins, kinin-like substances, protease and lysosomes (Crunkhon, 1971). It has been reported that second phase of edema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti- edematous effect of natural products (Schalm et al., 1975). The results of anti-inflammatory activities of various extract of bark of Crataeva religiosa was given in the Table -6.

Tripathy et al., (2010) studied acute and chronic anti-inflammatory potential of Crataeva religiosa in rats and concluded that the aqueous and alcoholic extracts of stem bark possess significant anti-inflammatory activity in rats.

The various extracts of bark of Crataeva religiosa was evaluated for analgesic activity against acetic acid-induced writhing in mice. All the extracts are having analgesic activity against the acetic acid induced writhing in mice but the activity of the ethanolic and aqueous extract shows significant activity than petroleum ether extract and chloroform extract. The reduction in writhing response are compared with the standard drug i.e. pentazocine and the result were shown in the Table -7.

Khatun farjana et al., (2012) reported that the leaves of medicinal plant ‘Crataeva nurvala’ Buch. Ham’ was extracted in ethanol to evaluate the peripherally acting analgesic potential using acetic acid induced writhing and antidiarrhoeal activity using intestinal motility test both in mice. The crude extract showed significant (P<0.01) analgesic activity at oral doses
of 200 and 400mg/kg body weight with an inhibition of writhing 68.4% and 76.3% compared to 67% for the positive control. In the motility test, the crude extract at same oral doses showed 31.16% and 35.31% inhibition of intestinal propulsion of charcoal marker where as positive control group exhibited 36.25% inhibition of propulsion of charcoal through the intestine.

**Antimicrobial Activity:**

Antimicrobial activity is the capacity of the substance to kill or inhibit micro-organism. The result of the present study indicates a strong antimicrobial activity of ethanol and aqueous bark extracts of *Crataeva religiosa* than aqueous and petroleum ether against the tested organism such as *Enterococcus fecalis, Escherichia Coli* and *Staphylococcus aureus*. The *Enterococcus fecalis* shows maximum zone of inhibition against the extracts than *Staphylococcus aureus* and *Escherichia Coli*.

The bactericidal activity of apical stem bark extract was maximum than meddle and mature bark against all the bacterial species were studied except the bacterium *Pseudomonas aeruginosa*. The bacterium *Staphylococcus aureus* was highly inhibited by apical stem bark extract (9.00 - 0.63mm) and *salmonella typhi* (5.500 - 0.77mm) respectively. The three bacterial species *Klebsiella pneumoneae, Proteus mirabilis* and *Micrococcus* species showed least inhibition zone in the range of 3.5mm’ at 300µl by apical stem bark extract .In case of *Pseudomonas aeruginosa*, mature bark extract (4.83- 0.41mm) and apical bark extract (4.17-0.41mm) had more similar inhibitory activity while the activity of meddle bark extract was minimum (3.83 -0.70mm) than these two. The extracts of apical and meddle bark were found ineffective at lower concentrations but effective in controlling the growth of all bacteria at higher concentrations. The extract of mature bark was ineffective in controlling the growth of
Klebsiella pneumoniae, Proteus mirabilis, Micrococcus species, Staphylococcus aureus and Bacillus subtilis at all studied concentrations while it inhibited the growth of Escherichia coli and Salmonella typhi showing least inhibition zones 2.67 -0.48mm and 1.33 -0.52mm respectively. The inhibition by negative control methanol was zero while the standard antibiotic chloramphenicol had inhibited the growth of all the bacterial species effectively at low concentration of 50u g/ml with the zone of inhibitions ranging from 11.17 mm to 21.50 mm as reported earlier by Patil, (2011).

The extract of Crataeva religiosa shows the significant (p<0.05) inhibitory effect on the formalin induced arthritis in a doseof dependent manner. After repeated induction of formalin on 3rd day after measuring the paw volume the organisms develop a chronic swelling. Standard diclofenac decrease the paw edema significantly from the first day of experiment. On the initial days extracts do not show significant effect which indicates its ineffectiveness in suppressing the immediate phase reaction induced by formalin (Tripathy et al., 2010).

Bijase et al., (2002) reported that three known isoflavonoids from methanolic extract of root bark and eight known isoflavonoids from the stem bark of Bolusanthus species which exhibits anti-bacterial activity against the Owalabi et al., (2007) reported antibacterial activity of ethanolic extract of kigelia africana stem bark against Staphylococcus aureus (15mm). Mann et al., (2008) noticed that the root bark, stem bark and leaves of Terminalia avicennioides extracted in ethanol exhibits bactericidal potential against Escherichia coli. Janovska et al., (2003) reported that Bacillus cereus, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans were inhibited by Chelidium majus, Sanguisorba officinalis and Tussilago farfara. Abu-saanab, (2004) investigated that the crude ethanolic extracts of Sanguisorba officinals, Sanguisorba aromaticum, Cinnamomum
verum, Rosmarinus officinalis, Thymus vulgaris, Sanguisorba officinalis / Rosmarinus officinalis / Thymus vulgaris inhibited the growth of multi drug resistant bacterium Pseudomonas aeruginosa. The bark extract was found to be contained in tannin glycosides, alkaloids, steroids and flavonoids which are biologically active (Shimada, 2006). Tannin from Dichrostachys cinerea root bark possesses anti-bacterial activities against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa (Mason and Wasseman, 1987). Flavonoids have been reported to have both anti-bacterial and anti-fungal activities (Tsuchiya et al., 1996).

Crataeva religiosa was selected based on its ethnomedicinal importance in Indian traditional system of medicines and the various extracts of the bark were screened invitro for their anti fungal properties. The results revealed that ethanolic extract among all extracts of Crataeva religiosa exhibited significant activity comparable to standard antifungal agents against all test fungal pathogens and their MICs (Minimum inhibitory concentration) were found to be 0.062-0.5mg/ml by two fold serial dilution method (Sahoo et al., 2008).

The different rates of inhibition may be probably due to the quantity of the phytochemicals present in the extracts (Mason and Wasseman, 1987). Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria (Lin et al.,1999; Parekh and chanda, 2006). These differences may be attributed to the fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure (Yao and Moellerling, 1995). The plant extracts inhibited the Gram positive micro-organisms better than Gram negative micro-organisms. This is in agreement with previous reports that plant
extracts are more active against Gram positive bacteria than Gram negative bacteria by Vlietinck et al., (1995).

Thus, it is important to know the biochemistry of stem bark in order to isolate and screen the new pharmacological active principals.

**Highlights of the work:**

- Pharmacognostical studies are focused on the identity of herbal drug by various methodologies as Macroscopy and Microscopical examination, ash values, extractive values and moisture content.

- Phytochemical studies investigate the various phytoconstituents such as alkaloids, sterols, flavonoids, carbohydrates, tannins, glycosides, saponins triterpenoids, proteins and amino acids by qualitative chemical analysis and thin layer chromatography.

- The anti-inflammatory and anti-analgesic potency of our drug are valuated by suitable experimental animal models.

- The anti-microbial activity of our medicinal plant *Crataeva religiosa* has been proved by agar diffusion method in various Micro-organisms.

### 8.0 SUMMARY AND CONCLUSION

The quest for good health and immortality has been a continuous human endeavour since the beginning of civilization throughout the World. In all ages and civilizations, man’s dependence on plants for food and medicine was well chronicled. Plants have continued to play a dominant role in the maintenance of human health since ancient times. Human beings are suffering from illness and diseases. The search for relief from ailments prompted them to explore