Summary and Conclusions

Plants are regarded as the pharmaceutical factories of natural origin for most of the drugs used by human beings. Plants medicines are highly important in the lives of human. As India is the largest producer and consumer of the medicinal drugs and is rightly called the botanical garden of the world (Dubey et al. 2000), most of the leading foreign pharmaceuticals companies are exploring the botanical garden of the world.

Among the many parts of plants that we use, bark accumulates large amount of medicinaly important phytochemicals such as phenols, sterols, alkaloids, saponins, terpenoids gycosides etc. Traditionally, bark products have been particularly prominent as sources of medicines and raw materials. The development of bark is different from the other plant parts. Bark drugs are usually dried parts of the medicinal plants that forms essential raw material for the production of traditional remedies of Ayurveda, Siddha, Unani, Homeopathy etc.

Different chemical compounds isolated from the bark exhibit wide pharmacological activities and plays role in treating the various disorders related to human health. Due to the high
demand of traditional remedies all over the world, there is danger of adulteration in the natural drug. Thus it is of prime importance to quantify the different chemical components of these phytodrugs which are helpful in detecting the quality and purity of the crude drugs.

Thus, taking into account all above aspects, the present investigation deals with the evaluation of some pharmacological properties such as antibacterial and antioxidant activities, estimation of various phytoconstituents, major organic and inorganic constituents and biotonic potential of the barks of medicinally important trees *Anogeissus latifolia*, *Crataeva religiosa*, *Pterocarpus marsupium* and *Terminalia arjuna*.

Significant findings of the present investigations are summarized as follows.

A. Antibacterial studies

The percent extract yield was higher during the summer season than the winter season. The bark of *T. arjuna* and *A. latifolia* shows higher extract yield than the bark samples of *C. religiosa* and *P. marsupium*. 
Bark extract of *A. latifolia*, *T. arjuna* and *P. marsupium* exhibited maximum inhibition zone (8mm). While, *C. religiosa* bark extract showed zone of inhibition (2.17mm) against the bacterium *B. subtilis*. Zone of inhibition of all the plant were lower than the control streptomycin (9.66 mm) and Chloramphenicol (21.50 mm) against *B. subtilis*.

The bark extract of the *A. latifolia* (11 mm) and *T. arjuna* (10 mm) and *P. marsupium* (8 mm) extracts were found to posses high bactericidal potential against *S. aureus* which was greater than reference antibiotics. Antibacterial activity increased with increasing concentration of the bark extract while *C. religiosa* extract of rind (9 mm) showed a higher zone of inhibition than inner and outer bark.

Bark extract of *A. latifolia* showed maximum inhibition zone against *E. coli* (11.50 mm) followed by the *T. arjuna* (8 mm) and *P. marsupium* (3.50 mm). *C. religiosa* showed minimum zone of inhibition (2.33 mm). Zone of inhibition of all the plant extracts were lower than the control streptomycin (14.17 mm) and Chloramphenicol (16.50 mm). It indicates that the extracts of *A. latifolia* and *T. arjuna* can be employed to control infection caused by *E. coli*.

The bark extract of *A. latifolia* and *T. arjuna* showed maximum inhibition zone 11.50mm and 11.0mm respectively against *P.*
*aureginosa* which was higher than the inhibition zone exhibited by *C. religiosa* (4mm) and *P. marsupium* (8mm) and Streptomycin (10.67mm) but lower than the Chloramphenicol (14.33 mm).

The methanolic extracts of *A. latifolia* showed higher zone of inhibition (14 mm) than other extracts, while, rest of the plant exhibits lower inhibition zone than these two plants. Standard antibiotics Streptomycin and Chloramphenicol showed 10.50 mm and 16.67 mm Zone of inhibition respectively. The bacterium *S.typhi* was less sensitive to the bark extracts of *C. religiosa* and *P. marsupium*.

All the bacterial species were highly sensitive to the methanolic bark extract of *A. latifolia* followed by *T. arjuna, P. marsupium* and *C. religiosa*. The methanolic extract of apical stem bark showed maximum inhibition than the inner and outer bark extract. The high bactericidal potential of *A. latifolia* was might be due to higher concentration of secondary metabolites or due to the presence of one or group of bactericidal compound dominating the secondary metabolite pool than the other bark samples. The lowest inhibitory potential of *C. religiosa* might be associated with lower concentration of secondary metabolites.
A. Biotonic studies

1. Germination studies

The lower concentrations stimulated the seed germination while higher concentrations were found inhibitory to the germination of the mung and wheat seed.

2. Seedling biomass and mobilization efficiency

All the concentrations of bark extract of *A. latifolia* and *T. arjuna* reduced the fresh weight of mung seedling while, *C. religiosa* and *P. marsupium* increased the fresh weight of the mung seedling at lower concentrations. The bark extract of *C. religiosa* upto 2% and *P. marsupium* at all studied concentrations increased the fresh weight of wheat seedling. The bark extract of *A. latifolia* and *T. arjuna* reduced the mobilization efficiency of the Mung seedling at all concentration studied and increased at lower concentrations of *C. religiosa* and *P. marsupium*.


Shoot length of mung seedling was stimulated at lower concentrations of aqueous bark extract of *C. religiosa* and *P. marsupium* while the bark extract of *T. arjuna* and *A. latifolia* decreases...
the shoot length with increase in concentration of the bark extract. All the bark extract increases the shoot length of wheat seedling up to 1% extract concentration whereas, the *P. marsupium* stimulated shoot length up to 5% bark extract concentration. The root length of mung seedling was adversely affected by all the concentrations of the bark extract. The root length of wheat seedling remains more or less stable at lower concentration (0.25%) of all the bark extract, except the bark extract of *T. arjuna*. At lower concentrations *C. religiosa* and *P. marsupium* stimulated the root length of wheat seedling. The bark extract of *A. latifolia* and *T. arjuna* reduced the mobilization efficiency of the mung seedling at all concentrations studied and increased at lower concentrations of *C. religiosa*, *P. marsupium*. All the bark extracts showed decrease in the vigour index of the mung seedling. *C. religiosa* at lower concentration (0.25%) showed slight increase in the vigour index. The bark extract of *A. latifolia* and *T. arjuna* negatively affected the vigour index of the wheat seedling in response to all studied concentration while, *C. religiosa* up to 2% and *P. marsupium* due to all studied concentrations increased the vigour index of wheat seedling.

Aqueous extract of bark of *T. arjuna* and *A. latifolia* inhibited shoot length, root length, reduced fresh weight vigour index and mobilization efficiency of mung seedling than the wheat seedling due
to the *C. religiosa* and *P. marsupium* bark extract. Similar observations have been made in case of wheat seedlings but, *C. religiosa* and *P. marsupium* positively influences the shoot length than mung bean. Both *T. arjuna* and *A. latifolia* contains high amount of total phenolic content as compared to *C. religiosa* and *P. marsupium*. The inhibitory effects by these combrataceae trees might be ascribed to their high phenolic content and might contains one or more above mentioned growth inhibiting allelochemicals indicating negative allelopathy (Huxley, 1999), while less inhibition observed by *C. religiosa* and *P. marsupium* might be due to their low phenolic content and absence of deleterious phenolic in the bark extract exhibiting positive allelopathy (Fernandez *et al.*, 2006). Stimulatory effects observed might be due to the fact that the one or more endogenous phenolic compounds acting as hormone analogues and affecting the plant physiology positively. Thus by defining a proper concentration of bark extract or by isolating growth stimulatory compound and intensifying its growth promoting concentration, the bark extract can be used for healthy plant growth as biotonic. While, the bark extract of *A. latifolia* and *T. arjuna* show inhibitory action on seed germination and seedling growth also displayed its inhibitory effects on bacterial growth.
C. Qualitative and Quantitative Studies on Secondary metabolite

Phytochemical analysis of barks of the four plants revealed that presence of alkaloids, flavonoids, flavones, glycosides, phenols, tannins, terpenoids, and saponin. A thin layer chromatographic analysis of terpenoids revealed that the maximum number of terpenes were noticed in rind of *T. arjuna* followed by *P. marsupium*, *A. latifolia* and *C. religiosa* than inner and outer bark of all the four plants. The presence of these compounds may play a role as the antibacterial phytochemical compounds.

Among all the four plants studied *Terminalia arjuna* showed higher levels of polyphenols, followed by *A. latifolia* *P. marsupium* *C. religiosa* in summer season and winter seasons. As stage of development advances decline in the polyphenol contents was noticed in all plants. In summer season polyphenols recorded were higher showing stimulated secondary metabolism in response to heat stress due to summer season.

Flavonoid content in the bark tissue was elevated in *A. latifolia* in summer season and winter season as compared to the other plants studied. The flavonoid contents also showed development related
decrease. In rind of young stem flavonoid content was elevated than other stages, showing stimulated secondary metabolism for the protection of young growing plant tissues from the summer shocks than the cooler winter seasons.

Tannin content in the plants also showed elevated in the summer season tannin the winter season in all the plants studied. A. latifolia and T. arjuna showed stimulated secondary metabolism in both summer and winter seasons.

The alkaloid content in the apical rind of these plant species was higher than the inner bark and outer bark. It was also noticed that the alkaloid content during summer season was higher than the winter in all the plant under study The concentration alkaloid varies in the order of accumulation A. latifolia > T. arjuna > P. marsupium > C. religiosa .

Thus the presence of secondary metabolites in young apical rind, inner bark and mature outer bark of A. latifolia, C. religiosa , P. marsupium and T. arjuna can enrich to improve its pharmacognostic active principles.

D. Antioxidant Potential of Bark Extracts

The antioxidant activity during the summer season is higher than the winter season. In Both seasons, in all plant samples (apical rind,
inner bark and outer bark), the antioxidant activity noticed is lower than the standard ascorbic acid and the lower levels of antioxidant activity was more prominent in the bark tissue of *C. religiosa*. Thus the phenolic content, flavonoid content, terpenoids content, saponin content and other polyphenolic compound with potential antioxidant activity from the bark *T. arjuna* and *A. latifolia* might have contributing to the free radical scavenging activity which was observed maximum than the *P. marsupium* and *C. religiosa* showing minimum polyphenolic moieties responsible for their low antioxidant activity. Thus all the bark (*A. latifolia*, *C. religiosa*, *P. marsupium* and *T. arjuna*) contain more or less phenolic compounds with varying level of antioxidant activity and can serve as good sources of natural antioxidant, and can be employed to overcome the toxic effects due to synthetic antioxidants.

The total antioxidant activity was greater during summer season than winter. In both seasons, apical rind of all the plants show highest antioxidant activity than inner and mature outer stem bark. Further, it was also noticed that the total antioxidant activity of *T. arjuna* was higher than the other bark samples and standard ascorbic acid. In apical rind, along with complex phenolic compounds, the concentration of the simple phenolic might be high which was showing high antioxidant activities than the inner and mature outer bark those might containing both phenolic compounds in lower amounts.
Reducing capacity of bark samples was higher in summer than the winter. All bark samples shows reductive ability in the order of preference- Apical rind > inner stem bark > mature outer bark. It was also noticed that the reducing power of the *T. arjuna* bark elevated significantly than the other bark samples as well as standard ascorbic acid. Thus, in both season all the three bark samples of *T. arjuna* had highest reducing power than the other three plants and *C. religiosa* exhibited lowest reducing capacity by mature inner bark in winter.

Nitric oxide scavenging activities of methanolic extract of bark of four medicinal plants *A. latifolia*, *C. religiosa*, *P. marsupium* and *T. arjuna* are significantly differing. Decreased absorbance of reaction mixture indicates that these plants have the ability to scavenging nitric oxide radicals. It was evident that the nitric oxide scavenging activity was maximum in summer than the winter. The pattern of inhibition of nitric oxide radical by methanolic extract was in the order- Apical rind > inner bark > Mature outer bark. In both season the maximum inhibition of nitric oxide is shown by apical stem bark than the inner and mature outer bark of all four plants. Our result indicates that decreased absorbance by addition of methanolic extracts of all the barks may contain the substance those are capable to inhibit the nitric oxide and thereby play positive role in human body against inflammatory conditions. The presence of nitric oxide scavenging principle in
significant amount explains the frequent use of these medicinal plants in the indigenous system of Indian medicinal preparation to treat the variety of disease conditions.

It was observed that inhibition of hydroxyl radical was higher during summer than winter. In both the seasons, in each plant, methanolic extract of apical stem bark exhibits higher radical scavenging activity than the inner and mature outer bark. Presence of phenolic compounds in bark might be responsible for their potent antioxidant potential. Diversity of different polyphenolic compounds with differential concentration in methanolic extract of different bark samples might be reflecting the differential hydroxyl radical scavenging antioxidant activities.

H$_2$O$_2$ scavenging activity was higher during summer than winter. Methanolic extract of all the bark samples exhibits quiet high hydrogen peroxide radical scavenging activity than standard control ascorbic acid (20.44%). In both summer and winter, peroxide radical effect shows higher in case of apical rind of four plants studied. Our results suggest that at concentrations 10 µg/mL all plants exhibited the significant hydrogen peroxide scavenging activity higher than the ascorbic acid. The dietary phenols protects mammalian and bacterial cells from cytotoxicity induced by H$_2$O$_2$ (Nakayana, 1994) indicates that the
observed $\text{H}_2\text{O}_2$ scavenging activity of our plants could be due to presence of phenols which can donate electrons to $\text{H}_2\text{O}_2$ neutralizing it to water. The decomposition of $\text{H}_2\text{O}_2$ by the extracts may form its antioxidant and free radical scavenging activity.

Methanolic extract of bark of all plants exhibits marked ferrous iron chelating activity which is quiet higher than the standard ascorbic acid. The maximum chelating activity was noticed in the summer than the winter season. Among all bark samples, methanolic extract of apical rind of each plant show highest metal chelating activity than the inner bark and mature outer bark. In both season, methanolic extract of all bark had potential to chelate ferrous ion which was higher than the standard ascorbic acid (45.05%) except the premature and mature inner bark of $\text{T. arjuna}$ had lower potential than the ascorbic acid. It indicates that in both seasons, at low concentration of 50µg/mL, all bark extract showed good antioxidant potential which is mostly higher than the standard ascorbic acid. All the bark samples studied show presence of chelating agent which is responsible for forming $\delta$ bonds with metal and stabilizing their oxidized form. These bark samples when employed as crude drug may work as natural iron cheaters for human health. The concentration of the secondary metabolites in the different bark samples is higher in the summer season than the winter season. Thus, summer season is suitable while harvesting the bark of
medicinal plants to meet the maximum availability of the bioactive compound and hence the bioactivity.

E. Organic Constituents

Starch amylose and amyllopectin content during winter season was higher than the summer season. There was appreciable accumulation of starch in the *A. latifolia, P. marsupium and T. arjuna* than *C. religiosa*. Further, winter season appears more suitable for accumulation of starch in the bark tissue. Further studies on properties of bark starch will throw more light on its further application into food, pharmaceutical and plastic industries.

The apical rind and inner bark contains higher level of total sugar content than the outer bark. Reducing sugars were elevated during summer season in all the bark samples. Sugar contents of all the bark samples were elevated during summer season while starch, amylose and amyllopectin contents elevated during winter season. This decrease in starch content during summer season may be due to reduced photosynthetic activities than the winter season. Increase in total sugars, might be associated with the osmoregulation of the bark tissue because dehydration and desiccation of the bark tissue results in the heat stress, which might be one of the reason for increasing levels of sugar contents and decreasing starch contents during summer season.
The crude fiber content of *Crataeva religiosa* was 2 times greater than the *A. latifolia, P. marsupium* and *T. arjuna* bark samples. Crude fiber content of apical rind, inner bark and outer bark was showed similar levels during both the seasons.

The total nitrogen and crude protein accumulated during summer season was higher than the winter season. The young apical rind show higher levels of nitrogen than the outer bark. This is more prominent in the bark of *P. marsupium*.

**F. Inorganic constituents**

The ash content of outer bark was higher than the inner bark and apical rind. Among these barks *T. arjuna* bark showed higher ash content.

The calcium content during summer season was higher than the winter in *A. latifolia, C. religiosa, P. marsupium* and *T. arjuna* bark. The calcium content of *T. arjuna* (2.31%) was significantly higher than the *A. latifolia, C. religiosa* and *P. marsupium*. The phosphorus content was significantly higher in apical rind of *C. religiosa* and *P. marsupium*. While, the phosphorus content was elevated in summer season and this elevation is noticeable in *C. religiosa* in apical rind, inner and outer bark. The magnesium content during summer season was higher than the winter season. The magnesium content in apical, inner and outer bark.
bark was uniformly distributed. The iron content in apical rind, inner and outer bark of *C. religiosa* was elevated during summer season. In *A. latifolia, P. marsupium* and *T. arjuna* Fe content was more or less unaltered in both the seasons. The manganese content of apical rind of *C. religiosa* and *P. marsupium* was significantly higher during winter season while the opposite trend was noticed in inner and outer bark samples. Whereas in *A. latifolia* and *T. arjuna*, the Mn content was elevated in the summer season than the winter season. The copper content of *A. latifolia, C. religiosa* and *T. arjuna* was higher during summer season while, in *P. marsupium* higher level of copper during winter season was noticed and during winter season. The zinc content of apical rind of *C. religiosa, P. marsupium* and *T. arjuna* was elevated during winter season. While the Zn content in other species *A. latifolia* and *T. arjuna* remains more or less similar in both the seasons. The chromium content was higher in bark of *C. religiosa, P. marsupium* and *T. arjuna* during winter season and the level of chromium was elevated in *P. marsupium* and *T. arjuna* when compared to *A. latifolia* and *C. religiosa*. The cadmium was not detected in *P. marsupium* bark during winter as well as summer season. The cadmium concentration in apical and mature outer bark was lower than the inner bark in *T. arjuna, A. latifolia* and *C. religiosa*. The nickel content was higher during winter
season. This was decreased significantly during summer season in *T. arjuna*, apical rind, inner and outer bark.

**G. Antinutritional Factors**

The nitrate content was higher during summer season than the winter season. The nitrate content was significantly higher in the bark of *A. latifolia* and *T. arjuna* than the bark of *C. religiosa*. This accumulation of the nitrate in the bark may as storage might be associated with the retranslocation under nitrogen deficient conditions and become available for further assimilatory process and may used during the unavailability of the nitrogen. The oxalate content was higher in summer season than the winter season. The oxalate content in the bark of *A. latifolia* and *T. arjuna* was higher than Oxalate content in the bark of *C. religiosa* and *P. marsupium*.

The bioactive compounds of these barks with their antimicrobial and antioxidant potential might have potential as therapeutic agent for the control of common bacterial infections of plants and animals. Further, these bark samples with these various organic and inorganic constituents can be utilized for development of ecofriendly bioactive compounds with least residual effects and may beneficial for the desining of residual free drug in near future.