6. SUMMARY AND CONCLUSION

Infectious diseases are one of man’s oldest enemies. They continue to be a serious burden around the world, in developing and industrialised countries alike. It is said that every decade produces its own pattern of diseases. Modern medicines consist mostly of antibiotics and chemotherapeutic drugs. Still the problems of microbial resistance are growing. Multidrug resistance towards the antibiotics and their related effects has an added effect in pursuit of the use of natural drugs. Given the evidence for rapid global spread of resistance and clinical isolates, the need for discovery of new antimicrobial agents is of paramount importance.

Free radical-mediated oxidative stress is believed to be the primary cause of many disorders, such as cardiovascular diseases, brain dysfunction, cataract, diabetes mellitus, arthritis, cancer and ageing. In the treatment of these diseases, antioxidant therapy has gained utmost importance in the recent years. Plants are a major source for the discovery of new products of medicinal value for drug development. Different strategies including the use of in vitro system, have been extensively studied to improve the production of plant chemicals.

Plants are the basic source of knowledge of modern medicine. Almost all the parts of the plant, namely leaves, flowers, fruits, bark, roots, stem and seeds are known to have various medicinal properties. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobials and antioxidants.

Higher plants have been shown to be a potential source for new antimicrobial compounds. As plants are commended as potent biochemists, man is able to obtain from them a wondrous assortment of industrial
chemicals. As the World’s human population continues to explode, there will be widespread of new microbial disaster. So, studies pertaining to the antibacterial and antioxidant properties of the plants need to be more concentrated.

One such medicinal plant used in the present study is Couroupita guianensis. Even though the plant parts are used in traditional medicine against several disorders, especially those against microbial infections, no systematic study on the nature of the antimicrobial action and the phytochemical responsible for this action has been reported. The study was formulated in four phases. In the first phase, the antibacterial and antifungal activity of various parts of the Couroupita guianensis namely, leaves, bark, flowers and fruit pulp were evaluated. In Phase II, the levels of enzymic and non-enzymic antioxidants in the plant parts were determined, followed by their radical scavenging ability and biomolecular protective effects. In Phase III, the phytochemical fractions present in the plant were isolated and assessed for their antimicrobial activity and free radical scavenging potential. The phytochemical fractions were also subjected to spectral analyses. In the last phase, the efficacy of the identified phytochemicals were checked by an in silico approach against the most susceptible microorganisms.

The results of Phase I clearly demonstrated that the methanol extracts of Couroupita guianensis flowers and fruit pulp exhibit broad spectrum antimicrobial activity. The intensity of the antimicrobial action varied depending on the microorganism. The flowers and fruit pulp of Couroupita guianensis showed equal effectiveness against both Gram positive and Gram negative bacteria and effective inhibitory effect against some fungal strains, followed by the leaves.
The second phase involved the analysis of the enzymic and non-enzymic antioxidants in the leaves, flowers and fruit pulp of *Couroupita guianensis* and the ability of these plant parts to scavenge a battery of free radicals. The antioxidant property of *Couroupita guianensis* was also checked using an *in vitro* model namely goat liver slices subjected to oxidative stress using H$_2$O$_2$.

It is evident from the results that the *Couroupita guianensis* flowers, fruits and leaves exhibited good antioxidant effect and strong scavenging effects on a battery of radicals and oxidants (DPPH, ABTS, H$_2$O$_2$ and OH$^-$) as well as strong protective effect on cellular biomolecules (lipids and DNA) subjected to oxidative stress. The results also confirmed that the *Couroupita guianensis* plant parts can improve the antioxidant status in oxidatively stressed tissues, which strengthens the antioxidant potential of the plant.

The next phase involved the identification of the phytochemicals by qualitative phytochemical analysis, followed by the spectral studies like TLC, HPTLC, UV absorption, HPLC, IR and GC-MS in order to identify the active principle present in the plant. The results of qualitative analysis indicated the presence of alkaloids, phenolics and flavonoids. In order to further confirm the role of the secondary metabolites, the alkaloid, phenolic and flavonoid fractions were isolated and checked for their antimicrobial activity and antioxidant potential. The isolated phytochemical fractions namely alkaloids, phenolics and flavonoids were also assessed for their radical scavenging ability against DPPH, ABTS, H$_2$O$_2$ and hydroxyl radicals.

Our results indicated the antioxidant potential of alkaloid, phenolics and flavonoids of the leaves, flower and fruit pulp of *Couroupita guianensis*. However, the antimicrobial activity was strongly associated only with the
alkaloid fraction, with the phenolic and flavonoid fractions exhibiting negligible activities. Thus, it is perceivable that the antimicrobial component in the plant is an alkaloid.

HPTLC fingerprinting also revealed the presence of alkaloids, phenolics and flavonoids in the flowers of *Couroupita guianensis*, indicating the type of active principles present in the plant. HPLC analysis confirmed the presence of five major peaks in the methanolic extract of the flowers of *Couroupita guianensis*. The IR spectrum of *Couroupita guianensis* confirmed the presence of polyphenolics and alkaloids in the flower sample.

The combined analysis of the spectra of the flowers of *Couroupita guianensis*, unequivocally confirmed the presence of alkaloids, phenolics and flavonoids. The analysis of the biological activities of these fractions revealed the antimicrobial activity to rest in the alkaloid fraction and a strong antioxidant potential in the phenolic and flavonoid fractions.

The IR and GC-MS spectra strongly indicated the structures of the active principle to match those of isatin and indirubin. These two active components were subjected to *in silico* studies to analyse their efficacy against the target proteins of the most susceptible organisms namely the bacterial strains *Shigella flexneri* and *Staphylococcus aureus* and the fungal strain of *Candida albicans*. The structure of the target proteins namely VirA (*Shigella flexneri*), MgrA (*Staphylococcus aureus*), SAP2 and SAP5 (*Candida albicans*) were obtained from the Protein Data Bank. The molecular docking and ADME studies were performed to characterize the active components. It is clearly evidenced that the two compounds possessed good docking scores and reasonable stability. The ADME profile supports the bioavailability of the compounds.
The *in silico* study provides evidence for the interaction of isatin and indirubin from *Couroupita guianensis* with the bacterial and fungal virulence-causing target proteins. This interaction is presumably vital in exerting the antimicrobial activity.

The results of the present investigation, thus, clearly demonstrate the antimicrobial and antioxidant activity of *Couroupita guianensis*. The antimicrobial effects rest predominantly with the alkaloid fraction, while the antioxidant potential is mainly exhibited by phenols and flavonoids. The study provides further evidence of the antimicrobial activity of the alkaloids by demonstrating their *in silico* interaction with the target molecules involved in the virulence of the organisms.

Thus, the present study strongly iterates the medicinal value of the plant, *Couroupita guianensis*, and scientifically validates it for use as a component of medicinal preparations, not only to combat pathogenic attack, but also to address the myriad diseases caused by oxidative stress.

**SUGGESTIONS FOR FUTURE RESEARCH**

The outcome of the present study has opened the way for addressing several other research problems in the current scenario. Some of the suggestions for the future research include the following:

- The phytochemical fractions may be purified further to isolate the single active component from the fractions and its structure can be elucidated.

- The active component can be subjected to clinical trials to develop into a novel drug.
The active components can further be docked against the other target proteins of the most susceptible pathogens of the present study.

The lead compounds can also be screened for their efficacy against the other bacterial and fungal strains.

The active components can be structurally modified \textit{in silico} and a QSAR (Quantitative Structure Activity Relationship) analysis can be done to identify the most potent derivative.