SUMMARY
Macrophages are the first line of defense against the invading pathogens provided by our immune system. Out of the various cells involved in immune response macrophages are considered to play an important role in mediating immune response by activation. Macrophages perform three basic function for combating the pathogen invasion i.e. Phagocytosis, antigen processing and release of different cytokines along with the production of reactive nitrogen and oxygen species. On the basis of this, different BRMs were used to activate macrophages.

Macrophages perform a variety of complex microbicidal functions, including surveillance, chemotaxis, phagocytosis and destruction of targeted organisms. The spectrum of microorganisms are kept on check by phagocytes include fungi, bacteria and virus infected cells (Beutler, 2004). Recent evidences suggest that development of preventative strategies to resist disease could certainly be a more efficient and possibly more effective longterm healthcare strategy (Hackett, 2003; Lollis and Bucala, 2003). The development of novel therapeutics, which could nonspecifically augment the innate immune response, represents an ideal strategy for addressing current worldwide concerns of how to combat classical and emerging infectious agents.

The effect of the BRMs on the activation of quiescent resting macrophages was assayed by its secretory activity of factors like nitric oxide (assayed in the form of nitrite) and lysozyme which leads to the microbicidal activity of macrophages. The alcoholic and aqueous extracts of \textit{T. cordifolia} have been tested successfully for immunomodulatory properties (Thatte et al., 1987; Dahanukar et al., 1988; Thatte and Dahanukar, 1989; Rege et al., 1989, 1999; Dikshit et al., 2000; Manjrekar et al., 2000). Previous authors have reported findings that most of the therapeutic compounds in plants are polar in nature and require polar solvents for their extraction. However, the ethanolic extract failed to show significant activity in the present study. The herb is being consumed by people since a long time. It would therefore suffice to extrapolate the \textit{in vitro} observations to \textit{in vivo} conditions. There is thus a great need to understand how exactly pathogen survives \textit{in vivo} conditions and identify pathways unique to the intracellular environment that could be utilized for the development of new and better drugs.

The study reveals that when macrophages were treated with different BRMs like gallic acid, guduchi, canova, cisplatin, AOIM-Z, spirulina and/or LPS they show significantly increased reactive oxygen ($\text{H}_2\text{O}_2$, $\text{O}_2^-$) and nitrogen intermediates;
enhanced enzyme levels like lysozyme, NADH and NADPH oxidase and myeloperoxidase; increased microbicidal and tumoricidal activities as compared to untreated macrophages. The destruction or killing of foreign particles as well as intracellular organisms depends on the cell’s capacity to secrete microbicidal metabolites of oxygen (Adams and Hamilton, 1984). On stimulation with appropriate agents in vitro the macrophages get activated and result in enhanced production of reactive oxygen metabolites (Warful and Zucker-Franklin, 1986). We could publish the reports that guduchi or LPS treatment can activate the macrophages to produce enhanced reactive oxygen metabolites ($\text{H}_2\text{O}_2$, $\text{O}_2^-$) and reactive nitrogen intermediates; enhanced enzyme levels like lysozyme, NADH-NADPH oxidase and myeloperoxidase; increased microbicidal and tumoricidal activities as compared to untreated macrophages (More and Pai 2011, 2012). Lysozyme, which is secreted constitutively and in large amounts by macrophages, was found to be further increased in BRM treated macrophages. Lysozyme is highly active against grampositive species and deficiency in lysozyme production has been found to lead to susceptibility to Streptococcus pneumoniae (Shimada et al., 2008). Though the role of $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ in the tumoricidal activity of macrophages is not clear but several correlations have been indicated (Hall et. al., 1983). It was observed that culture supernatants obtained from BRM treated macrophages show enhanced tumoricidal activity which increases depending upon the duration of treatment and this was parallel to the production of $\text{H}_2\text{O}_2$ and $\text{O}_2^-$. Therefore it is possible that $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ may play a significant role in the tumoricidal process, either alone or in combination with other tumor cytolytic factors produced by activated macrophages (Geetha and Sodhi, 1989). Superoxide anions dismutate to hydrogen peroxide, which is converted to HOCl by the peroxidase. The interaction of HOCl with superoxide anions then generates hydroxyl radicals. In parallel NO interacts with superoxide anions and generates apoptosis-inducing peroxynitrite. Signalling by reactive oxygen and nitrogen species seems to represent a hitherto unrecognized signaling principle for the selective elimination of potential tumor cells by macrophages (Heigold and Bauer, 2002).

It is well known that activated macrophages secrete a number of cytolytic factors among which monokines, IL-1 (interlukin-1) and TNF (tumor necrosis factor) are important in the tumoricidal activity (Ichinose et. al., 1988). It was observed that BRM or LPS stimulated macrophages produced enhanced TNF in the culture medium.
suggested that macrophages produce a plethora of tumoricidal molecules including the ROS. Other investigators have also reported that macrophage or macrophage like cell lines when stimulated with PMA or MAF show increased capacity to lyse the tumor cells *in vitro* by a mechanism involving release of oxidative intermediates. (Hall *et. al.*, 1983) Nathan also reported that H₂O₂ can rapidly lyse a variety of neoplastic cells (Nathan, 1979). Lysozyme is considered to be one of the constitutive enzymes of mononuclear phagocytes (Gordon *et al.*, 1974). Macrophages treated with BRMs for 24 and 48h release significantly more lysozyme as compared to untreated macrophages. LPS treatment also showed similar pattern. Gordon *et al*.1974 reported that BCG stimulated rabbit alveolar macrophages have high levels of intracellular lysozyme. They suggested that this increased lysozyme secretion is associated with substantial net synthesis of a relatively constant intracellular and continuous accumulation in the medium. Macrophages when appropriately activated shows enhanced microbicidal and tumoricidal effects. When activated by BRMs the product nitric oxide (NO) is generated upon conversion of L-arginine into L-citrulline, a reaction catalysed by the enzyme-inducible nitric oxide synthase (iNOS) (Green *et al*. 1982; Green *et al*., 1990; James, 1995). Production of NO appears to constitute one of the main microbicidal mechanisms of murine macrophages (Liew, 1990) and has been implicated in the elimination of viruses, bacteria, fungi and protozoa including *Leishmania* (Degroote *et al*., 1995; Stenger *et al*., 1994). TNF-α can be directly cytocidal to tumor cells *in vitro* and it augments tumoricidal activity of macrophages. Activation of macrophages to tumoricidal state requires signal transduction, gene transcription and translation (Kunkel *et al*., 1986; Kunkel *et al*., 1986; Kunkel *et al*., 1988) and includes production of antitumor molecules like IL-1 and TNF (Browning *et al*., 1987; Phillip and Epstein 1986). Kreigler *et al*. (1988) suggested that activated monocytes synthesize transmembrane TNF at the site of inflammation and kill their targets by either cell to cell contact or local release of the TNF secretory component. There are reports that monocytes elicit TNF in response to contact with tumor cells (Webb *et al*., 1990; Janicke and Mannel, 1990). We therefore investigated if BRM treated macrophages would produce enhanced amounts of TNF as compared to untreated macrophages. The killing of tumor cells by culture supernatants of macrophages treated with BRMs might be due to a combined action of TNF and other cytolytic factors. Ruggerio and Baglioni (1987) have shown that IL-1 and TNF act synergistically and additively in killing...
tumor cells (Ruggerio and Baglioni, 1987). There are accumulating reports on the generation of superoxide radicals or other reactive oxygen intermediates (ROI) that are capable of damaging cellular components and activating the release of certain enzymes that might be involved in the TNF-initiated cytotoxic pathway (Ruddle, 1987; Smolen 1984; Jones, 1986). True to this report, we have also observed enhanced ROI production by macrophages after BRM treatment. The study suggests that treatment of macrophages with the BRMs or LPS can render them cytotoxic to L929 and Yac-1 cells by release of ROI and TNF in the culture medium. Activated macrophages can kill tumor cells by two mechanisms, one via contact mediated cytolysis and the other in which the activated macrophages release cytolytic factors in the culture medium (Sodhi et. al. 1990; Pai and Sodhi, 1991; Sodhi and Singh, 1986; Sone et. al., 1985). We suggest that one mechanism by which BRMs or LPS treated macrophages kill the tumor cells is manifested by the production of TNF.

Ayurveda remains one of the most ancient and yet living traditions practised widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis (Dahanukar, 2000; Chopra and Doiphode, 2002). *Atharvaveda* (around 1200 BC), *Charak Samhita* and *Sushrut Samhita* 26 (1000–500 BC) are the main classics that give detailed descriptions of over 700 herbs. A scholarly description of the legacy of Caraka in contemporary idiom, best attempted with a commentary from modern medicine and science viewpoint, gives some glimpses of ancient wisdom (Valiathan, 2003). Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions (Waxler-Morrison, 1988). India has about 45,000 plant species; medicinal properties have been assigned to several thousands. About 2000 are found in the literature; indigenous systems commonly employ about 500–700. Some recent work in drug development relates to species of *Commiphora* (used as a hypolipidaemic agent), *Picrorhiza* (which is hepatoprotective), *Bacopa* (memory enhancer), *Curcuma* (antiinflammatory) and *Asclepias* (cardiotonic) (Jain, 1994). Currently, with over 400,000 registered ayurvedic practitioners, the Government of India has formal structures to regulate quality, safety, efficacy and practice of herbal medicine (National Policy on Indian Systems of Medicine and Homoeopathy, 2002). With unique holistic approach, ayurvedic medicines are usually customized to an individual constitution. Exhaustive information is available in ayurvedic literature that can be converted into a large database giving information of various foods (Amadea
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Moringstar, 1990), herbs, medicines and other materials with their taste, actions and utility in different disorders.
The dynamic process of evolution could alter and affect the identity and structure of natural materials. Additionally, some botanical species might have been extinct. Lastly, the properties of botanicals as recorded in classics may have undergone change due to time and environmental factors. Therefore standardization of ayurvedic botanicals and medicines is required. Recently, many international authorities and agencies, including the World Health Organization (WHO, 2000), European Agency for the Evaluation of Medicinal Products and European Scientific Cooperation of Phytomedicine (EMEA/CVMP, 2001), US Agency for Health Care Policy and Research (FDACDER, 2000), European Pharmacopoeia Commission, Department of Indian System of Medicine have started creating new mechanisms to induce and regulate quality control and standardization of botanical medicine. For ayurvedic medicine and other traditional medicines, newer guidelines of standardization are required. A botanical drug or a preparation thereof is now regarded as one active substance in its entirety, whether or not the constituents with therapeutic activity are known. This will be a major step in the development of new generation standardized botanical medicines. The WHO has published official documents on medicinal plants and WHO monographs on selected medicinal plants (WHO, 1996). Global definitions of botanical products are being developed with international cooperation and a new perspective of standardization, validation, safety and efficacy of botanical medicines is evolving – this is a good sign.

Phytochemicals has large range of activities and one of them is immunomodulation. Experienced based work is being carried out since long time and its time to make it experimental, so the work is done on plants based on nature products. In this study we have tried to overcome the Doctor’s dilemma that ‘don’t provide drugs but stimulate the macrophages (phagocytes) because it’s the nature’s remedy.