Discussion
The various forms of traditional systems of medicine, using plants as the basis of health care have evolved in different ethological, cultural, climatic and geographical regions of the world. Among these systems, Ayurveda, one of the ancient traditions of Indian system of medicine occupies unique position. About 7,500 plants have been included and used in local health traditions in mostly, rural and tribal villages of India. Out of 7,500 plants, about 2000 plants have been prized for their medicinal properties (Kumar et al., 2011; pushangadan, 1995). However, despite the use of herbal medicines over many centuries, only a relatively small number of plant species has been studied scientifically in detail for possible medical application. Further, there are also a substantial number of "negative monographs" narrating risk from active ingredients or absence of reasonable proof of efficacy (Keller 1992). Therefore, to make it competitive and acceptable, and to give a better defined status to phytotherapy and herbal remedies there is a need of scientific validation with vigorous attempts to prove their genuine effectiveness required to treat the ailments of humans and animals or to promote defence mechanisms against pathogens. This can be achieved by following the line approach of evidence as adopted in modern medicine and understanding of supportive and allied subjects depicting the role of biomolecules (Cytokines & Chemokines) in regulating the defense mechanisms involving innate and adaptive immune systems and the immune homeostasis of host encircled by hostile environment.

To accommodate the potential of plants in drug approval data WHO recommended developing pharmacopial monographs on herbal medicines. The information in the monograph should include two parts: Part I consists of the botanical characteristics, major phytochemicals and quality control of each plant, Part II comprises clinical applications in terms of safety and toxicity, pharmacology, posology, contraindications and potential adverse effects. An innovative research effort to define the merits of traditional
systems of medicine with respect to their safety and efficacy in terms of antimicrobial activities and immunomodulation could result in better utilization of complementary system of medicines (WHO 1991, 1994, 2003).

*C. fistula* occupies significant position in the indigenous system of medicine of many Asian, African and South American countries (Bahorun et al., 2005). It is also known as Amalts, Aragvadha (disease killer) and Golden shower. Besides *C. fistula*, other species of *cassia* such as *C. alata, C.sophera, C. javanica C. auriculata* plant have also been studied (Abo et al., 2000; Ayo 2010). Although the plant has a high therapeutic value and reported to possess hepatoprotective, anti-inflammatory, antitussive, antifertility, antimicrobial and antitumor activities, (Rizvi et al., 2009; Danish et al., 2011; Yadav and Jain, 1999), but, perhaps little information is available regarding its therapeutic value in terms of its role in immunomodulation and in regulating cytokine biomolecules. Therefore, following the WHO guidelines, study has been framed to find out the medicinal potential of *C. fistula* plant with scientific evidences.

Prior to the study, three extracts (hot aq. extract of pods, HAEP; hot aq. extract of leaves, HAE and hot hydromethanolic extract of pods, HMEP) were prepared and subjected to preliminary qualitative phytochemical testing. During analysis it was found that glycosides and reducing sugars were present in all the three extracts, but varied in other phytochemicals. Alkaloids, tannins and phenolic compounds were present in HAE and HMEP but not in HAEP, while flavonoids and proteins were present in HAEP and HAE (table 10). This study shows that different extract preparations contain phytochemical in varying proportion. Luximon-Ramma et al. (2002) studied that pod contained the highest level of phenolic compound and phenolic flavonoid and proanthocyanidin contents in the leaves. Vasi and Kalintha, (1980) estimated the protein and carbohydrate contents in the fruit. Panda et al. (2011) evaluated phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and aminoacids, saponins and triterpenoids in polar extracts compared with non polar extracts of *C. fistula*. 
All the extracts were also analyzed for the presence of phytochemicals by thin layer chromatography and HPLC and revealed that HAEP and HAEL had three similar Rf values (0.87, 0.125, 0.156) while HAEP exhibited 0.262 Rf value in addition. HMEP showed total six spots with Rf values: 0.87, 0.156, 0.175, 0.212, 0.262, 0.362. Rf values and spots were taken as number of phytochemicals in the extract. Rf values 0.87, and 0.156 were same in all extracts is indicative of same compounds, while three same compounds are present in HAEL and HMEP indicated by an additional Rf value 0.262, which did not find in HAEP (fig.1, table 13). All the extracts were also analyzed by HPLC for the separation and identification of compounds. The identification of the compounds present in the extracts was based on the comparison of obtained peaks at similar RT of standards and extracts. Results indicated that peak with RT of 2.11min. was present in all the extracts which was present at similar RT of quercetin dehydrate. Similarly peak obtained at RT 7.5min. indicated the presence of kaempferol in HAEL (fig. 7). Peak obtained at RT 3.8 and 3.3 showed the presence of gallic acid and sinapic acid in HMEP (fig. 8) Comparison of the retention times of the individual component showed that there are variations in regard to retention times. However, this type of variation has been reported in literature (Strack and Reznik, 1979; SamAsan, 1984). All the results obtained by different methods (qualitative tests, TLC, HPLC) used for the phytochemicals analysis of three extracts indicated that these extracts are differ in phytochemical proportion.

The first objective of this work has been to determine the novel dose that could be safe, well tolerated, nontoxic with minimum adverse reaction or side effects and amendable at least for oral administration or a topical application. So the three doses (125/250/500mg/kg body weight) of aq. extract of HAEP / HAEL of *C. fistula* were given orally to respective groups of albino rats for 21 days. At regular intervals, change in health status, body weight and development of any adverse effect were recorded. All the three doses of HAEP and HAEL were found safe and nontoxic as no ill effect was
observed in any of the treated groups of rats. All the treated animals exhibited a normal trend in body weight gain (table 17).

To provide support to this work hematological estimation of treated rats was also conducted (table-18). The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status of humans and animals (Jothy et al., 2011). Both HAEP and HAEEL fed rats exhibited increase in PCV and Hb values. But higher Hb values (16.19gm/dl ± 0.191, 16.39gm/dl ±0.0202, 16.1gm/dl ±0.232) were recorded in the groups of HAEEL fed rats when compared to control group (13.2 gm/dl ±0.386) and all the HAEP fed rat (15.48, 15.68, 15.66gm/dl) groups (fig. 10). Erythrocytosis and leucocytosis were also pronounced in experimental rats fed with any dose of either extract. An increase was also noticed in percentage of lymphocytes (68.4±1.029, 65.2±1.496, and 67.6±0.892) in HAEEL and (65.6±0.4, 67.0±0.84, 66.2±0.72) in HAEP fed rats in respect to unfed rats 63.4±1.326 (table 18, fig. 9). This study revealed that extract of leaves and pods of *C. fistula* might be hematopoietic effect, and immunogenic property. Protein estimation was also carried out, but there was no change in the protein content in the serum in treated rats in relation to rats of control group (table-19). Treated rat did not exhibit any symptoms of acute as well as chronic toxicity. However, in contrast to our result Jothy *et al.* (2011) have observed no change in the hematological parameters in rats after the treatment of *C. fistula* seed extract. However, Many other workers (Bhakta *et al.*, 1999; Kannampali *et al.*, 2005; Das *et al*; 2008; Patwardhan *et al.*, 2009; Jahangir *et al.*, 2010.) have worked on other parameters like effect on AST, ALT, ALP, SGOT and SGPT.. Akanmu *et al.* (2004) have calculated 6600 mg/kg body weight as LD$_{50}$ dose of aqueous extract of *C. fistula* pods in albino rats. Ali *et al.* (2008) have showed that treatment up to the concentration of 1gm/kg body weight of aqueous extract of pod did not reveal any adverse effect in mice. Nontoxicity of different parts of *C. fistula* was also observed by given orally for 7 to15 days and could not find adverse
Discussion

effect Raju et al. (2005) Ziaurrahman et al. (2011). Jothy et al., (2011) have found that seed extract at dose of 5000 mg/kg could not produce any ill effect. Other species of cassia were also studied by many scientists for toxicity. Sangetha et al. (2008) have studied that C. spectabilies leaf extract at 2000 mg/kg body weight did not produce adverse effect. Selvi et al. (2010) found that administration of aq. extract of C. auriculata seeds (2000mg/kg) for 28 days did not show any chronic as well as single dose of 5000mg/kg did not produce acute toxicity in rats. Our observations and their findings confirmed the non toxicity of pods and leaves extracts of C. fistula as well as of other species of cassia studied in different countries. On the basis of results obtained dose of 500 mg/kg body weight and below were taken as safe and non toxic doses for further study.

Moduloregulation of immune system by using herbal medicines may be possible alternative to conventional chemotherapy. Several studies find phototherapy as evidenced by traditional systems of medicine including Ayurveda could be helpful in healing the variety of idiopathic diseases arising due to impaired immune responses. Presently on account of emergence of countless number of allergens (pollutants) and limitations of modern medicines in elimination of immunological disorders, use of medicinal plants having immunomodulatory or immunoregulatory effects appears to be more beneficial (Patwardhan and Gautam, 2005).

Keeping these in mind, in this investigation immunological activity of HAEP and HAEL of C. fistula was evaluated by determining the humoral and cell mediated immune responses. Humoral response was studied by measuring antibodies by agglutination test using Salmonella typhimurium 'O' antigen and results were compared with control. It was observed that antibody titer in the serum samples of all the treated rats was higher in relation to control. Further, it was noticed that HAEL fed rats exhibited much higher antibody titer (358.4±62.71, 409.6±62.71, 409.8±51.2), over the serum antibody titer (153.6±25.6, 256.0±42.7 256.0±42.6) rats (table 20, fig. 11) measured in HAEP fed groups. This study shows that rise in humoral
response may be due to the stimulation of B-lymphocytes, TH cells and macrophages subsets by pods and leaves extracts. It is well established fact that B lymphocytes participate in antibody formation (Makare et al., 2001, Sehar et al., 2008).

Effect of HAEP and HAEL of *C. fistula* on cell mediated immune response was also examined by skin hypersensitivity reaction using DNCB as an allergen. All the doses of both extracts exhibited a marked enhancement in skin thickness. Rats fed with 250 mg/kg body weight of HAEP showed maximum increase in skin thickness by 27.12%/21.11%/14.49% at 24/48/72 hours respectively. While rats treated with 125 mg/kg body weight of HAEL induced increase in skin thickness by 33.15% / 22.67% 16.33% at 24/48/72 hrs respectively (table 21, 22, fig.12, 13). This finding revealed that HAEL in comparison to HAEP was more effective in stimulating CMI and humoral responses. The augmented humoral and cell mediated immune responses in the presence of HAEP and HAEL of *C. fistula* reflect the proliferation and activation of both B and T lymphocytes. Literature surveyed showed that some work has been carried out on the immunomodulatory activities of *C. fistula* and other species of *cassia*. Ali et al. (2007) have investigated that mice treated with water extract of fruit (pod) of *C. fistula* and *amoxy-cassia* (Synergistic effect) enhanced humoral response by activating large number of antisheep red blood cells. Das et al. (2008) have studied that patients treated with *C. fistula* pulp showed a significant decrease in serum IgE level producing significant relief in patients suffering from eczema or Leprosy. Chakraborty, (2009) have found that *C. auriculata* caused a significant stimulation of cell mediated immunity but no effect on humoral response. Tilwari et al. (2011) have noticed the immunostimulatory activity of *C. tora*. From the results of present study, it was observed that *C. fistula* had stimulatory influence on humoral and cell mediated immunity as evident from increased antibody titer against *S. typhimurium* 'O' antigen and significant enhancement in skin thickness in DNCB hypersensivity. Our results were based on the number of repetition of
experiments and showed that extracts of pods (HAEP) and leaves (HAEL) might have inducted the stimulation of B and T cells both. Our preliminary phytochemical studies showed the presence of alkaloids, carbohydrates, tannins and phenolic compounds, which have been reported to have immunomodulatory activities (Tilwari et al., 2011).

Study was further carried out to study the extent of proliferation of splenocyte using spleen of albino rat model. Spleen is a major secondary lymphoid organ that responds to blood born infection and trap antigen and contributes to humoral as well as cellular arm of immune system. Therefore for determination of effect of HAEP and HAEEL on splenocytes, *in-vitro* experiments were designed in albino rat model. It was found that HAEP and HAEEL had significant stimulatory effect on splenocytes. Both extracts exhibited dose dependent induction of Con-A treated splenocyte proliferation (fig.14, 15, 16). 62.5µg/ml, 125µg/ml, 250µg/ml, and 500µg/ml of HAEP/HAEEL enhanced 9.76%, 13.48%, 26.97%, 33.02% / 6.04%, 11.16%, 36.28%, 37.29% proliferation respectively (table 23). *Ex vivo* experiments using splenocytes harvested from HAEP extract fed (125/250/500mg/kg body weight) rats exhibited 11.87%, 18.06% and 9.03% spleen cells proliferation respectively (table 24, fig. 17, 18). On the basis of results obtained by the study of hematology, immune responses and splenocytes proliferation experiments it is concluded that extracts of *C. fistula* might have increased B and T cells populations, key factors for humoral and cell mediated responses. Such information perhaps has not been reported by any other worker working on *C. fistula*. However, John *et al.* (2011) working on *Cassia auriculata* another species of *Cassia* plant demonstrated proliferative effect on T and B lymphocytes along with proliferation of splenocytes.

It is now well known that cytokines, low molecular protein play major roles in inducting and regulating the development of effective immune responses involving cells of immunocytes, inflammatory and haemopoietic systems (Kubey, 2003). Presently more than 200 different cytokines have
been described and the uses of cytokines offers the prospects of specific chemical therapies to modulate immune responses in organ transplantation, infectious diseases and allergy; inflammation and cancer therapy (Kubey, 2003). So keeping the clinical significance of cytokines in mind and to support the results of immunomodulation by HAEP and HAEL so far obtained, the two important cytokines: IFN-γ and IL-10 produced by TH1 and TH2 subsets of helper T cells respectively were taken for study of their induction in the presence of HAEP and HAEL of C. fistula. This study may offer an explanation of the effect of C. fistula on the immune system. Our observation during this study revealed that HAEP and HAEL enhanced the IFN-γ production using splenocyte culture in relation to control. While, there was no more or less change in the concentration of IL-10 under the influence of HAEP / HAEL of C. fistula. IFN-γ and IL-10 are two cytokines produced by two different TH1 and TH2 subsets of T helper cells (TH, CD4 cells and have antagonistic effect. It means up regulation of IFN-γ inhibit the proliferation of TH2 thus down regulating the IL-10 induction and vice versa (Kubey 2003; Roitt, 2001).

In vitro experiments were conducted in rat model using 62.5 µg, 125µg, 250µg and 500µg/ml of HAEP/HAEL of C.fistula and dose dependent IFN-γ induction was recorded. 250µg/500µg of both HAEP and HAEL induced 29.33% / 34.71% and 35.21% / 37.40%, higher induction of IFN -γ respectively. However, 62.5/125 of both HAEP and HAEL produced 12.07% / 11.13% and 8.46% / 6.87% increase in IL-10 production and these same concentrations induced 13.27%, 19.06%, 9.56% and 16.60% increase in IFN-γ production (table 25, 26, fig. 19, 20). However, in our study, there was no down regulation of IL-10 in conjunction with up regulation of interferon gamma. To say in confirmation, this study requires more efforts in detail.

For study of antibacterial activity of C.fistula along with HAEP and HAEL, HMEP was also attempted. Three well established bacteria (Staphylococcus aureus, Esherichia coli, Pasteurella multocida) were used. Five concentrations: 625mg/1.25mg/5mg./10mg./20mg./disc was employed.
All the three extracts exhibited dose dependant antimicrobial activity against *S. aureus* and *Past. multocida*. Maximum inhibition zone (15.2mm) was noticed with 10 mg HAEP while 0.625 mg/disc the lower most concentration could not show inhibitory effect against *S. aureus* (table 28, fig. 21). There was no inhibitory action of any of the three extracts of *C. fistula* against *E. coli* (table 29). Various workers (Vimalraj *et al.*, 2009; Abbas *et al.*, 2004; Growther and Janardhan, 2010; Vasudeven *et al.*, 2009) have reported good antimicrobial activity of different extracts of different parts of *C. fistula* against *S. aureus*. Similar to our findings Vimalraj *et al.*, (2009) and Sangetha *et al.*, (2008) observed no activity of extracts of pods and leaves of *C. fistula* against *E. coli*. However, in contrast to our findings Bhalodia *et al.* (2011) have noticed effect against *E. coli*. Vimalraj *et al.*, (2009) have showed no inhibitory effect of extracts of *C. fistula* against *past. multocida* but in our study all the extracts (HAEP, HAEL and HMEP) demonstrated effective anti *Past. multocida* activity (table 29, fig. 22). On prolonged incubation, bacterial colonies reappeared within the zone of inhibition indicating bacteriostatic effect rather than bactericidal activity.

Mycotic pathogens are always a challenge to human and animals and very difficult to control. Though, the antifungal drugs are available but these drugs are very costly. In this investigation all the three extracts of *C. fistula* were also tested against *C. albicans*, and only HAEL showed antifungal activity against *C. albicans* in dose dependent manner (table 31, fig. 23). Higher concentration 20mg/disc of HAEL produced highest inhibition zone. Many scientists have reported (Lachumy *et al.*, 2010; Panda *et al.*, 2010; Bhalodia *et al.*, 2011; Aneza *et al.*, 2011) anti fungal activity of different extracts of different parts prepared from *C. fistula* against *C. albicans*. Phongpachit *et al.* (2004) have observed that the leaves extracts of *C. fistula* had 100% anti fungal activity against *Tricophyton rubrum*, *Microsporum gypsum* and *Penicillium rubrum*. Sangetha *et al.* (2008) have reported that *C. albicans* was susceptible to leaves, flowers, stems and pods extracts of *C. fistula*. These antimicrobial reports, may be attributed due to presence of
phytochemicals such as flavonoids, glycosides, tannins and phenolic compounds extracts and are effective in inhibiting the growth of microbial population (Panda et al., 2011). Other species of cassia i.e. C. tora, C. alata, C. javanica have been also reported to have antimicrobial potential. (Thambidurai et al., 2010; Khan et al., 2001; Mohtar and Sharri, 2000).

Unlike antimicrobial drugs against bacteria and fungi, only a few antiviral drugs are available. The lack of success in developing antiviral drugs is due to the nature of the infectious viral agents, the different modes of replication which totally depend upon the cell they infect for their replication and survival, so that many compounds that may cause the death of viruses also are very likely to injure the host cell that harbor them (Montanha et al., 2004). These problems urge the need to develop antiviral agents. In an effort to discover new compounds, the hot aqueous extracts of pods and leaves of Cassia fistula were also screened against Infectious Bovine Rhinotracheitis (IBR) virus using MDBK cell line. For this non toxic dose (MNTD) at which no any degenerative change /CPE found in cell culture was determined (table-32), .625, 1.25, 2.5 and 5 mg/ml of hot aqueous extract of both extracts were found to be non toxic dose(s), as there was no CPE of MDBK cell line (fig. 24, 25).

The antiviral activity of these non toxic concentrations was evaluated by assaying inhibition of CPE in MDBK cell cultures challenged with 10TCID$_{50}$ dose of IBR virus (table 33, fig. 26). It was seen that extract prepared from pods of Cassia fistula has pronounced antiviral activity. The dose of 5/2.5 mg/ml inhibited the CPE by 89/73%, when compared to IBR virus infected cell line (control) while 44%, and 33% reduction in CPE was recorded using 2.5, 1.25 and .625 mg/ml of pod extract respectively (table 34, fig. 27). This study also showed dose dependent antiviral activity. During this study HAEL could not exhibit antiviral activity and there was complete destruction of cells observed in MDBK cell line challenged with TCID$_{50}$ (fig. 28). Perhaps there is no availability of literature indicating the antiviral property of pods extracts of C. fistula. However, other species of Cassia
have been screened for their antiviral potential. Recently, Kitazato et al. (2007) have reported that extract of *C. angustifolia* has been found effective against HSV-1 (herpes virus). Hussein et al. (1999) El Mohamed et al. (2010) have noticed antiviral activity of *C. obtusifolia* against HIV-1. Ethanolic extract of fruit of *C. fistula* exhibited antiviral effect against Foot and Mouth Disease Virus (Narong et al., 2007). This experiment was repeated three times and results were consistent. From the results it is clear that pod of *Cassia fistula* has antiviral activity.

On the basis of results achieved in this study, it may be concluded that herbal medicines too have significant characteristics which distinguish them from the chemical drugs; use of crude extracts and prolonged usage. A single herb may contain a many natural constituents and these constituents work in conjunction with each other. Further, systems like Ayurveda still need to gain an empirical support of modern medical science to make them credible and acceptable for all. This kind of research work to define the advantages of traditional systems of medicine with respect to their safety and efficacy could result in a better utilization of complementary system of medicine in treating ailments of human beings and animals suffering with infectious and immunological disorders.
Summary and Conclusions
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Viewing the review of literature regarding the therapeutic potential of *C. fistula*, the present study was undertaken to study the antimicrobial and immunomodulatory properties of the pods and leaves of this plant with reference to their effect on IFN-γ and IL-10 regulation. Hot aqueous extract of pods and leaves (HAEP and HALE) of *C. fistula* were used for the study. Hydromethanolic extract (HMEP) of pods was also used for antibacterial and antifungal activities. First of all, extracts were prepared and subjected to preliminary qualitative phytochemical testing. During analysis it was found that glycosides and reducing sugars were present in all the three extracts (HAEP, HALE and HMEP) but varied in showing other phytochemicals. Alkaloids, tannins and phenolic compounds were present in HALE and HMEP but not in HAEP, while flavonoids and proteins were present in HAEP and HALE. All the extracts were also analyzed for the presence of phytochemicals by thin layer chromatography and revealed that HAEP and HALE had three similar Rf values (0.87, 0.125, 0.156) while HAEP exhibited 0.262 Rf value in addition. HMEP showed total six spots with Rf values i.e. 0.87, 0.156, 0.175, 0.212, 0.262, 0.362. All the extracts were also analyzed by HPLC for the separation and identification of compounds. Results indicated that peak with RT of 2.11 min. was present in all the extracts which was present at similar RT of quercetin dehydrate showed the presence of this compound in each extract. Similarly peak at RT 7.5 min. indicated the presence of kaempferol in HALE. Peak obtained at RT 3.8 and 3.3 min. showed the presence of gallic acid and sinapic acid in HMEP. All the tests performed showed that these extracts exhibited varying proportion in phytochemicals constituents.

Prior to investigate medicinal activities of HAEP and HALE of *C. fistula*, safe and nontoxic dose was determined by feeding 125mg/250mg/500mg HAEP/HAEL per Kg body weight to albino rats for 21 days. It was observed that all the fed rats did not show any change in their
behavior, skin, and fur texture and in food intake and water consumption. Experimental rats exhibited a slight increase in their body weight (20.42%, 24.23%, 20.60%, and 25.61% 22.81% 20.60%) in GI to GVII in respect of control (GI) group. All treated rats were also examined for hematological and serum protein analysis on 22\textsuperscript{nd} day. Both HAEP and HAEL fed rats exhibited increase in PCV (47.2, 46.4, 47.2, 47.6, 47.6, and 47.2) and Hb (15.48, 15.68, 15.66, 16.19, 16.39, 16.1) values when compared to control (45.2) (13.2). An increase was also noticed in percentage of lymphocytes in HAEP (68.4, 65.2, 67.6,) and HAEL65.6, 67.0, 66.2 fed rats in respect to control (63.4) rats. Serum protein analysis did not show any remarkable change.

To determine immunomodulatory effect of HAEP/HAEL of \textit{C. fistula} humoral immune response and cell mediated response were evaluated. Humoral response was measured by the serum antibody titer by agglutination test. There was a significant rise in antibody titer in HAEP/HAEL fed groups. Serum antibody titer in HAEP treated rats ranged from 153.6 ± 25.6 to 256.0 ± 62.71. Where as HAEL fed rats exhibited much higher antibody titer ranging from 358.4 ± 62.71 to 409.0 ± 51.2 when compared to untreated rats.

DNCB sensitized hypersensitivity test was conducted to evaluate the effect HAEP/HAEL on cell mediated immune response. 125 mg/kg fed rats induced maximum increase in skin by 33.15% at 24 hours 22.67% at 48 hours and 16.33 at 72 hours respectively. Rats fed with 250 mg/ml HAEP exhibited marked enhancement in skin thickness by 27.12% at 24 hours, 22.11% at 72 hours and 14.49% at 72 hours in respect to control group. But albino rats fed with 500 mg/kg induced less increase in their skin thickness in respect to rats fed with 125 mg/ of either extracts per kg body weight. This study revealed the T-cell stimulating effect of HAEP/HAEL of \textit{C. fistula}. Viewing the immunostimulant properties of HAEP/HAEL of \textit{C. fistula}, study was planned to determine the effect of both extracts on Con-A stimulated rat splenocytes and cytokine (IFN-\gamma and IL-10) induction. For this \textit{in vitro} and \textit{ex vivo} (for HAEP) were designed. For \textit{in vitro} study, four concentrations
(62.5µg/125µg/250µg/500µg/ml) of HAEP/HAEL of *C. fistula* were used and results indicated that both HAEP and HALE exhibited dose dependent induction of Con-A treated splenocyte proliferation. 500µg and 250µg of HAEP caused 33.02% and 26.97% proliferation in spleen cell culture respectively. 500µg/ml, 250µg/ml of HALE enhanced 37.20% and 36.28% splenocytes proliferation. Low degree stimulation of spleen cells was noticed on using of 62.5µg/ml, 125µg/ml of HAEP/HAEL of *C. fistula*. However, low induction of spleen cell proliferation was observed in *ex-vivo* experiment using spleens harvested from rats fed with 125/250/500 mg HAEP /kg body weight.

*In vitro* effect of HAEP and HALE of *C.fistula* on induction of cytokine IFN-γ and IL-10 was evaluated by sandwich ELISA using supernatant of 48 hours splenocytes culture. This study revealed that concentrations (500mg/ml and 250mg/ml) of both (HEAP and HALE) induced 37.40%, 35.21%, 34.71% and 29.33% increase IFN-γ production respectively. However, lower concentrations (62.5mg and 125mg) of any extract could not induced marked increase in IFN-γ cytokine production. Both extracts HAEP and HALE did not show any remarkable induction of IL-10 cytokine in relation to control. Lower concentrations of HAEP (62.5mg/ml and 125mg/ml) showed 12.07% and 11.13% while same concentrations of HALE produced 8.46% and 6.87% increase in IL-10 induction respectively.

To study the antimicrobial activity of HAEP, HALE and HMEP of *C. fistula* five different concentrations (1.25/2.5/5/10/20mg/disc) were used against well known pathogens i.e. *Staphylococcus aureus, E. coli, Pasteurella multocida, Candida albicans*. All extracts exhibited dose dependant antibacterial activity against *S. aureus* and *Past. multocida* while concentrations of both extracts used in this study could not produce any inhibitory effect against *E. coli*. HAEP of *C. fistula* also exhibited dose dependant antifungal activity against *Candida albicans* but HAEP and HMEP failed to show any static or cidal effect against *C. albicans*. 
Anti-infectious bovine rhinotracheitis virus (IBR) effect of (HEAP and HAEL) was also investigated using MDBK cell line. First of all Non-toxic concentration(s) of HAEP and HAEF was calculated and Concentration causing no damage to MDBK cell line was taken as non-toxic dose(s). Up to 5mg of HAEP and HAEF were found safe and non-toxic concentrations. TCID$_{50}$ (tissue culture infective dose) of IBR virus was calculated on the basis of development of degenerated changes in MDBK cells (cytopathic effect) and then finally antiviral activity of extracts was analyzed by using non-toxic concentrations of HAEP /HAEL in confluent 24 hours old MDBK cells challenged with 10TCID$_{50}$ of IBR virus. This study revealed that HAEP showed dose dependent anti-IBR activity, while HAEF of C. fistula could not produce any activity against IBR virus. A significant protection i.e. 89%, 72%, 43% and 22% was recorded with 5mg, 2.5mg, 1.25mg and 0.625mg/ml of HAEP respectively.

From the results observed in this study, it may be concluded that non-toxicity and presence of different secondary metabolites in pods and leaves of C. fistula provide scientific evidence to the ethnomedical use of this plant. This study also shows that hot aq. extracts of both pods and leaves have antimicrobial potential and can stimulate both humoral and cell mediated immune responses. Thus, further extract of C. fistula also have a property of modulation of IFN-γ and IL-10 cytokines. However, further studies are needed to better evaluate the potential of the plant as the antimicrobial agents and mechanisms of immunomodulation and probable use in immuno compromised individuals.