ABSTRACT

In the present study, phytochemical and pharmacological screening of two plants (*Rhododendron arboreum* and *Carum copticum*) was done. Alcoholic extracts of the flowers (ERAF) and leaves (ERAL) of *R. arboreum* and seeds of *C. copticum* (ECCS) were prepared by continuous hot percolation method using soxhlet apparatus. Aqueous (ARAF) extract of the flowers of *R. arboreum* was prepared by cold maceration method. Carbohydrates, steroids, phenolic compounds, flavonoids and saponins were identified in ERAF and ERAL. ARAF gave the positive tests for the above compounds except steroids. ERAL and ECCS were fractioned with n-hexane, chloroform and n-butanol by solvent-solvent extraction method. Preliminary immunomodulatory activity of the above fractions was evaluated by haemagglutination technique. n-Hexane fraction of both the extracts were found to be most active. Therefore, this fraction of both the extracts was selected for further isolation. Three terpenoids (ursolic acid, an unidentified terpinoid and 3, 10-epoxyglutinane), one sterol (β-sitosterol) and two flavonoids (Quercetin and Rutin) were isolated from the alcoholic extract of the leaves of the *R. arboreum* (ERAL) using column chromatography. Quercetin was isolated from the diethyl ether fraction of alcoholic extract of the flower of *R. arboreum* by solvent-solvent extraction method. Isolated compounds were identified and characterized by chemical tests, m.p., chromatography and spectroscopic methods. The flavonoidal compounds, quercetin and rutin, were simultaneously identified in flowers and leaves of *R arboreum* using high-performance thin-layer chromatography (HPTLC).

In the case of *C. copticum*, chemical tests of ECCS represented the presence of alkaloids, carbohydrates, steroids and phenolic compounds. Four oily sub-fractions (PC-b, PC-d, PC-g and PC-i) were isolated from n-hexane fraction of ECCS by column chromatography and one volatile oil (C-1) was extracted by steam distillation. Sub-fraction PC-b contained *p*-cymene, PC-d contained carvacrol, PC-g contained terpinene and PC-i was a dark-brown coloured oily liquid and contained unidentified components. Volatile oil (C-1) was extracted by steam distillation from the seeds of *C. copticum* using Clevenger apparatus. C-1 is a very light-yellow colored transparent liquid. It contains three major components, which were characterized by mass-spectrometry. Mass spectrum gave the peaks at m/z 135.12 (M+1 for *p*-cymene), 137.14 (M+1 for α-pinene) and 151.12 (M+1 for thymol). These three components were further
confirmed by HPLC. Out of the three, thymol, was found to be the major component. Therefore, the quantitative estimation of thymol was carried out in C-1 by GLC with an authentic marker. Structures of all the isolated compounds were elucidated by spectroscopic methods like IR, PMR, CMR and Mass spectrometry.

Immunomodulatory activity of ERAL and ECCS was performed against SRBC antigenic challenge model in mice. Three parameters (humoral immune response, cell mediated immune response and total leukocyte count) were used to evaluate the effectiveness of the extracts. The, orally administered ERAL showed a significant suppression of the immune responses, in a dose dependent manner. Physiology and liver function tests like total bilirubin, SGPT and SGOT of extract-treated mice were also statistically insignificant in comparison with levamisole and vehicle-treated mice. Thus, it can be concluded that the ERAL is an effective and safe immunosuppressive agent. Animals treated with different doses of the ECCS, showed an increase in the HA titers, DTH-response and phagocytosis in a dose dependent manner. The DTH-response of ECCS is a direct correlate of cell-mediated immunity (CMI). The test showed a significant increase in footpad thickness in 48hrs, as compared with the control and Levamisole (standard) treated group. DTH-response of the highest dose was found to be better than the medium dose. This indicated the overall stimulatory effect of ECCS on cellular immunity when compared with the levamisole treated group. The results of the in-vitro polymorphonuclear (PMN) function test (phagocytosis) showed a significant increase in the per cent phagocytosis at the doses of the 300 mg/kg and 500 mg/kg body weight.

Some other activities like anti-microbial, anti-tumor and anti-cancer of R. arboreum were also performed. Anti-microbial activity of the alcoholic and aqueous extracts and isolated quercetin was investigated against five bacterial and two fungal strains by agar well-diffusion method. The activity was found to be concentration dependent. Alcoholic extract was found to be more active in comparison to the aqueous extract. The lowest effective concentration of quercetin was found to be 12.5 mg/ml against S.aureus and P.aeruginosa. Both extracts and isolated quercetin were found ineffective against fungal strains.

Preliminary in-vitro anticancer screening of the alcoholic extracts of the flowers and leaves of R. arboreum was performed against Crown gall tumor and MCF-7 breast cancer cell lines. Both the extracts showed prominent inhibition in the development of Crown gall tumor in potato discs while only leaves extract was found to be significantly effective against MCF-7 cells.