PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF SOME PLANTS USED IN FOLKLORE MEDICINES.

A

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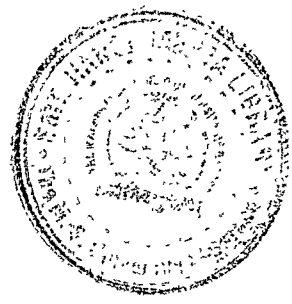

SUMMARY

Some plants are believed to promote positive health and maintain organic resistance against infection by establishing body equilibrium. It is tempting to speculate that the restorative and rejuvenating power of these herbs may be due to their action on the immune system. Traditional Indian systems of medicines like Siddha and Ayurveda have suggested means to increase the body's natural resistance to disease. A number of Indian medicinal plants and various 'rasayanas' have been claimed to possess immunomodulatory activity (Atal et al., 1986; Patwardhan et al., 1990; Puri et al., 1994).

In the present study, therefore three plants ***Sphaeranthus indicus***, ***Cissampelos pareira*** and ***Curculigo orchioides***, from traditional medicine, designated for their activity as a rasayana drugs were selected in order to establish the scientific data pertaining to immunomodulatory activity.

The selected plants were identified using standard texts and further subjected to pharmacognostic studies involving morphological and microscopical evaluation. Proximate analysis was carried out in order to standardize them. Further, these drugs were subjected to phytochemical screening. Successive solvent extraction carried out, suggested all the three plants contain maximum percentage of polar components. Out of these one, *C. orchioides* contains very less amount of non polar components as evidenced by lesser extractive values for non polar solvents. These drugs when subjected to identification of different class of chemical compounds present, *S. indicus* showed presence of terpenoids and flavonoids, *C. pareira* showed presence of alkaloids, saponins and phenolics, whereas *C. orchioides* showed presence of saponins, carbohydrates and phenolics. TLC profiles of the successive extracts were obtained in order to know the number and R_f of different phytoconstituents present.

The data obtained from preliminary experiments and literature available was utilized to select extracts for screening these drugs for biological activities. Methanol extract of all the three drugs was subjected to treatment with different solvents to collect different fractions. In case of *S. indicus*, fractions obtained were petroleum ether, benzene,

chloroform and methanol. Further, the water extract of S. *indicus* was fractionated into n-butanol and remaining water fractions. Methanol extract of *C. orchioides* was fractionated into hexane, chloroform, ethyl acetate and methanol. It was observed that non polar solvent yielded very less amount of the constituents; hence only methanol extract of *C. orchioides* was used for screening biological activity. Methanol extract of *C. pareira* was divided into alkaloidal and non alkaloidal fractions, as alkaloids from this plant were reported pharmacologically active agents. Thus, methanol extract and water extract and their different fractions of *S. indicus*, methanol extract of *C. orchioides* and methanol extract and alkaloidal fraction of *C. pareira* were screened for the biological activity in order to identify bioactive fraction.

Several plants when screened for immunomodulatory properties were found to have exerted it through antioxidant activity [La Fuente and Victor, 2000; Ruby et al., 1995 and Devasagayam and Sainis, 2002]. Many 'Rasayanas' drugs of Ayurveda were also reported to exhibit antioxidant activity (Scartezzini and Speroni. 2000). Therefore, the selective extracts of the selected drugs were also screened for antioxidant activity using *in vitro* models such as DPPH assay, superoxide assay, nitric oxide assay, reducing power determinations and in vitro lipid peroxidation, using rat liver homogenate.

All the test extracts were also screened for immunomodulatory activity using different reported methods in mice. The methods adopted were carbon clearance test, haemagglutination antibody titre, delayed type hypersensitivity and cyclophosphamide induced myelosuppression assay. The selection of method was based on the assessment of the effects on different arms of immunity like humoral immunity, cellular immunity, phagocytic function and bone marrow activity. The bioactive extracts so identified from above were further subjected to evaluation of immunomodulatory activity in cyclophosphamide (a potent anticancer drug), immunosuppressed animals using different treatment schedules.

The results of biological screening lead to identification of bioactive extract. The extract and /fractions having maximum activity were residual methanol fraction of methanol extract of *S. indicus*, methanol

extract of *C. pareira* and methanol extract of *C. orchioides* were screened for their protective effect against drug induced immunosuppression. In the present investigation two schedules of pretreatment period, i.e. 7 days pretreatment and 15 days pretreatment were selected. The results obtained in the present studies showed that bioactive fraction of *S. indicus*, methanol extract of *C. orchioides* and methanol extract of *C. pareira* displays a dose dependent immunostimulatory effects in relation to antigenic stimulation.

Studies on antioxidant activity of these drugs using some of the popular in vitro methods showed different levels of activities. Methanol extract and its residual methanol fraction of *S. indicus*, methanol extract and alkaloidal fraction of *C. pareira* and methanol extract of *C. orchioides* exhibited scavenging activity of DPPH. Methanol fraction of *S. indicus*, alkaloidal fraction of *C. pareira* and methanol extract of *C. orchioides* were strong scavengers of DPPH. Whereas, other tested extract and /or fractions of these plants hold only moderate activity.

The NBT (Nitro blue tetrazolium) reduction method showed that the methanolic extracts of *S. indicus*, and *C. pareira* possessed moderate, while bioactive fraction of *S. indicus*, alkaloidal fraction of *C. pareira* and methanol extract of *C. orchioides* were strong scavengers of superoxide radical generated in riboflavin-NBT-light system *in vitro* as compared to the standard, ascorbic acid.

The reducing power is associated with antioxidant activity (Duh et al., 1999). Methanolic extracts of *S. indicus*, and *C. pareira*, and *C. orchioides* possessed moderate reducing power, while bioactive fraction of *S. indicus* and alkaloidal fraction of *C. pareira* exhibited activity comparable to standard, ascorbic acid.

Methanol extract of *C. orchioides* only showed moderate activity in scavenging nitric oxide compared to standard Curcumin All the other extracts and /or fractions from *S. indicus* and *C. pareira* failed to show any scavenging effect against nitric oxide radical.

Inhibition of the lipid peroxidation induced by iron/ADP/ascorbate complex was noted with the extracts studied. However, strong activity was noted with bioactive fraction of *S. indicus*, methanol extract of *C.*

orchioides and alkaloidal fraction of *C. pareira*. Methanol extract of *S. indicus* and *C. pareira* hold the moderate activity.

From the above studies, the active extracts possessing significant biological activities and antioxidant activity were selected. HPTLC finger print profile was established for these extracts and /or fractions as HPTLC is now widely accepted as a potential tool for rapid and useful phytochemical evaluation of herbal drugs.

Bioactive extracts also subjected for isolation of components using recommended methods. Compounds isolated were checked for their homogeneity using different solvent system, different detecting agents. These compounds were then utilized for their characterization.

Petroleum ether extract of *S. indicus* afforded two components separated from unsaponifiable matter by using fractionation and column chromatography techniques. Petroleum ether fraction of methanol extract of *S. indicus* gave four compounds, isolated by using column chromatography. Bioactive residual methanol fraction of methanol extract of *S. indicus* afforded one compound. Two compounds were isolated from methanol extract of *C. pareira*. One compound was isolated from methanol extract of *C. orchioides*, but yield was too less to characterize it. These compounds were characterized using different physico-chemical as well as spectral studies.

The physiochemical data as well as data obtained from various determinations obtained from different compounds were recorded. The correct structure, however, could not be assigned for the want of detailed information.

Thus from the present study it can be concluded that-

- ***Sphaeranthus indicus, Cissampelos pareira*** and ***Curculigo orchioides*** certainly possess immunomodulatory (Immunostimulatory) activity as evidenced by stimulatory effects on humoral immunity (HA titre), cellular immunity (DTH response) and phagocytosis.
- ***Sphaeranthus indicus, Cissampelos pareira*** and ***Curculigo orchioides*** also provide protection against cyclophosphamide

induced immunosuppression of humoral immunity and myelosuppression.

- ***Sphaeranthus indicus, Cissampelos pareira*** and ***Curculigo orchioides*** also possess good antioxidant activity *in-vitro* as they are effective scavengers of DPPH, superoxide, nitric oxide and lipid peroxidation.

Thus, the present study can provide a valuable piece of information with regard to therapeutic effectiveness of these drugs as member of 'Rasayana' class. These studies, however, also provide further scope of detailed investigations on the mechanism of action and isolation and characterization of components from identified bioactive extracts so as to assign biomarker for the standardization of these drugs. In nutshell, the purpose of standardizing these drugs for their therapeutic effectiveness could achieve.

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extract/fraction could achieve statistically significant levels of HA titre when compared to control group (Table 3.10, Fig 3.11).

Methanol extract of the *C. pareira* was tested at five dose (50-800 mg/kg) levels for evaluating its effect on humoral response to sheep SRBC. The humoral antibody titre value in control was found to be 149.33 ± 35.70. Administration of methanol extract produced increase in humoral antibody titre as evident by haemagglutination at that dilution, but statistically significant increase couldn't obtained by any dose. Antibody titre at 50 mg/kg was 96.00 ± 14.11, 100 mg/kg was 128.00 ± 28.62, 200 mg/kg was 160.00 ± 32.00, 400 mg/kg was 181.33 ± 34.72 and 800 mg/kg 160.00 ± 32.00 (Table 3.11, Fig 3.12, A).

Alkaloidal fraction of *C. pareira* tested at four dose levels (25-100 mg/kg). Administration of alkaloidal fraction decreased level of antibody titre in dose dependent manner, however, higher doses had no effect on antibody titre compared to control. Alkaloidal fraction at 25 and 50 mg/kg, decreased levels significantly, 5.00 ± 1.00 ($p< 0.01$) and 8.00 ± 1.79 ($p< 0.01$) respectively. Further increase in dose was ineffective in decreasing the levels of antibody titre. Antibody titre at 75 and 100 mg/kg was 64.00 ± 14.31 (non significant) and 133 ± 40.83 respectively (Table 3.12, Fig 3.12, B).

Methanol extract of *C. orchioides* was screened at doses five doses ranging 50-800 mg/kg. Administration of methanol extract enhanced the antibody titre in dose dependent manner. However statistically significant increase could obtained at higher doses only. It showed levels of, 145.05 ± 40.20, 150.77 ± 35.04, 195.11 ± 25.22, 250.98 ± 11.20 ($p<0.01$) and 255.81 ± 18.90 ($p<0.01$) at doses 50, 100, 200, 400, 800 mg/kg, b.w. respectively (Table 3.13, Fig 3.13).