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EVALUATION OF IN-VITRO IMMUNOMODULATORY ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *CAPPARIS MOONII*

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ABSTRACT

The present study was intended to evaluate the *in-vitro* immunomodulatory activity of aqueous and ethanolic extract of dried fruits of *Capparis moonii Wight.* Effect of both the extracts were evaluated at various concentrations (832 µg/ml to 6.5 µg/ml) for secretion of variouesmediators like nitric oxide, superoxide, lysosomal enzyme etc of isolated murine peritoneal macrophages. Both the extracts showed in-vitro phagocytic stimulation of, lysosomal enzyme and myeloperoxidase activity and nitric oxide in peritoneal mouse macrophages. In-vitro phagocytic index showed significant results and thus proving the need for confirmation through in-vivo studies.

KEYWORDS:Capparis moonii, peritoneal murine macrophages, nitric oxide, superoxides, phagocytic index

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INTRODUCTION

The immune system is known to be involved in the etiology as well as pathophysiological mechanisms of many diseases (Sharma, 1983). Ayurveda gives emphasis on promotion of health – a concept of strengthening host defenses against different diseases (Thatte & Dahanukar, 1986). Rasayana plants are particularly recommended for the treatment of immune disorder (Wagner, 1999), Ayurveda (with particular reference to plants) may play an important role in modern health care, particularly where satisfactory treatment is not available. Development of agents, capable of moving patient's immune system from a state of immune deficiency to normal function, would likely to have a significant impact on patient's disease condition. . Such agent would not be a cure, but would control the manifestation and course of disease (Gottlieb et al. 1987).Plants belonging to family *Capparacea* have important medicinal properties and are distributed in tropical and sub tropical India. Plants belonging to this family have been described as a Rasayana herb and have been used extensively as an adaptogen to increase the non specific resistance and as immunostimulants. The *C sepiaria*, *C spinosa, C tomentosa* and *C zeylanica*, *C moonii* etc., belong to this family and are reported as used in traditional medicine (P Rajesh, a review on ……. *s*family).*CAPPARIS MOONII WIGHT (*belonging to family**:** *Capparidaceae/Capparacea)* commonly known as Large Caper, *Rudanti* in Sanskrit and Waghati in Marathi, is distributed in Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu. Large Caper is the largest flower among all Caper flowers. The main active compounds present in the fruits of *Rudanti* are sitosterol, stachyhydrin, rutin, Gallotannins (chebulinic acid derivatives).Therapeutic Uses of *Rudanti* in Ayurveda:are *Rudanti* nourishes each and every cell of the body (Rasayani). It is useful in under nutrition and emaciating conditions (Shoshghani). It delays the signs of aging (Jara Vinashnam) and is also useful in diseases which are having devastating effects on all the systems of the body (Rajyakshma Shasyate). *Rudanti* has also been extensively used to get relief from asthma and cough by the people of India. It exhibited significant antitussive activity as that of codeine phosphate, when compared with control in a dose dependent manner in sulfur dioxide gas induced cough model (P Sunita, S Jha., S. P. Patanayak). Bioassay guided fractionation of the hydro-alcoholic extract of the fruits of Capparis moonii, led to the isolation of two new chebulinic acid derivatives. The compounds 1 and 2 displayed significant glucose uptake effect of 223% and 219% over the control at the 10ng/ml and 100ng/ml concentration, respectively. The increased glucose uptake effects of the compounds were associated with significant IR and IRS-1 phosphorylation, GLUT4 and PI3-kinase mRNA expression in the L6 cells. (Lakshmi B. S., Kanaujia A) The effect of the ethanol extract of Capparis moonii Hook. f. Thoms. (Capparidaceae) fruits was studied in carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. The hepatotoxicity was induced in rats with the administration of 1:1 (v/v) mixture of CCl_4 in olive oil at the dose of 1 ml/kg subcutaneously on day 7. The ethanol extract of C. moonii (200 mg/kg) and the standard drug silymarin (25 mg/kg) were given orally from day 1 to day 9. The extract of C. moonii produced significant ($p < 0.001$) lowering of the elevated Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP), and a rise of depleted total protein when compared with the toxic control.

The results were comparable with the standard drug silymarin. (Prevention of carbon – tetra-chloride induced hepatoxicity by the ethanolic extracts of Capparis moonii in rats(Pharma Biology, 42:286(2004) According to the literature survey there is no reported research on immunomodulatory activity so our main objective was to explore immunomodulatory activity.

MATERIALS AND METHODS

Plant Material and Preparation of Extract

Fruits of *Capparis moonii*, were purchased from local suppliers in Jan. 2012 and authenticated from Agharkar Research Institute, Pune, India. The voucher specimen (No.F-176) was deposited in the herbarium of the Institute for future reference. The fruits were cut into small pieces and dried at controlled temperature 45^0C and powdered. The powder was then extracted with ethanol under soxhlation to give ethanolic extract of fruits of *Capparis moonii*, (CME) and similarly with water under soxhlation to give aqueous extract of fruits of *Capparis moonii*, (CMA). The extract were filtered and evaporated to dryness with a dryer.

Preliminary Phytochemical Screening

The ethanolic and aqueous extracts of *Capparis moonii* fruits were subjected to a Preliminary phytochemical screening (Trease and Evans, 1983) for the detection of various plant constituents.

Chemicals

Nitroblue Tetrazolium (NBT) and Tetramethyl Benzedrine Hydrogen Peroxide (TMB/H_2O_2) were procured from Bangalore Genei, India. Streptomycin, penicillin, Roswell Park Memorial Institute (RPMI) 1640 medium and HEPES buffer were procured from Himedia Pvt. Ltd. India. Fetal bovine serum (FBS) and Phytohemagglutinin-M (PHA) were procured from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Isolation of peritoneal macrophage and culture conditions

Peritoneal macrophages were isolated from mice which were injected intraperitoneally (i.p.) with 2 ml of 4% (w/v) fluid thioglycollate medium 3 days prior to peritoneal lavage with 10 ml of RPMI 1640 medium. The collected cells were washed with RPMI 1640 and cultured in RPMI 1640 supplemented with 10% FBS, 2mM L-glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin (complete RPMI). The macrophage count was determined by using haemocytometer and cell viability was tested by trypan-blue dye exclusion technique. Then the cells were adjusted to required cell count per ml and plated on a 96-well flat-bottom culture plate (Tarsons Products Pvt. Ltd., India) and then incubated for 2 hr at 37^0C in a 5% CO_2 humidified incubator. After removing the nonadherent cells, the mono-layered macrophages were treated with both the extracts separately (832-6.5µg/ml) dissolved in complete RPMI medium containing 20% DMSO and maintained for 24 hrs at 37^0C in a 5% CO_2 humidified incubator (Choi C. Y, Kim J. Y. …Cancer letter 166, 2001, 17-25).Following *in vitro* assays were performed on these incubated cells. PHA (10µg/ml) was used as a positive control. All the experiments were performed in triplicate.

Nitrite Assay

Nitrite accumulation was used as an indicator of nitric oxide (NO) production in the medium as per the procedure described earlier(Lee S., Suk K…\.pharmacology 74, 723-729). Cell-free supernatant (50µl) from 24 hrs incubated macrophages ($5x10^5$ cells/ml) was mixed with 50µl of Griess reagent (1% sulfanilamide, 0.1% napthylethylenediamine dihydrochloride, and 2% phosphoric acid) and incubated at room temperature for 10 min. The microplate reader (ELX800MS, BioTek Instruments Inc., USA).was used to measure the optical density (OD) at 540 nm. A standard curve of sodium nitrite in culture conditions was used to determine Nitrite concentrations. Stimulation index (SI) for nitrite release was calculated as the nitrite concentrations ratio of the treated and control macrophages.

NBT Dye Reduction Assay

The NBT dye reduction assay was carried out as described previously (Manosroi A., 2006). Briefly, 50µl of 0.3% NBT solution in PBS (phosphate buffered saline, pH 7.4) was added to the 24 hr incubated cells ($1×10^6$ cells/ml) with CMA & CME extracts separately, and the mixture was further incubated in CO_2 incubator. After incubation for 1hr, the adherent

macrophages were rinsed vigorously with complete RPMI medium, and washed four times with 200µl Methanol. After air-drying, formazan-deposits were solubilized in 120µl of 2M KOH and 140µl of DMSO. After homogenization of the contents of the wells, the OD was read at 630 nm by using a microplate reader. Stimulation index (SI) was calculated as the OD ratio of the treated and control macrophages

Cellular Lysosomal Enzyme Activity

The cellular lysosomal enzyme activity of macrophages was evaluated by measuring acid phosphatase activity as described earlier (Manosroi A., 2006). Briefly, 24 hrs after incubation of macrophages with CMA & CME extracts separately at 37°C in 5% CO_2, the supernatant was removed by aspiration and 20µl of 0.1% Triton X-100 (Himedia, India) were added to each well. After 15 minutes incubation, 100 µl of 10 mM *p*-nitrophenyl phosphate (*p*NPP) and 50 µl of 0.1 M citrate buffer (pH 5.0) were added. Further the plates were incubated for 1 hr and 0.2 M borate buffer (150 µl, pH 9.8) was added. The OD was measured at 405 nm by using a microplate reader. The Phagocytic stimulation index (SI) was calculated as the OD ratio of the treated and control macrophages

Myeloperoxidase Activity Assay

Myeloperoxidase activity was evaluated on isolated macrophages as per the earlier procedure (Choi EM., Hwang J.K., 2004). Briefly, 24 hr incubated macrophages ($5x10^5$cells/ml) with test extracts (CMA & CME separately) were washed three times with fresh complete RPMI medium. Then the mixture of *o*-phenylenediamine (0.4g/ml) and 0.002% H_2O_2 in phosphate-citrate buffer (pH 5.0) was added to each well. The reaction was stopped after 10 min using 0.1 N H_2SO_4 and OD were measured at 490 nm. The myeloperoxidase stimulation index (SI) was calculated as the OD ratio of the treated and control cells.

Statistical Analysis

Results are expressed as Mean ± SEM. Data was analyzed by one way ANOVA followed by Dunnets Multiple Comparisons Test. P value less than 0.05 was taken as the criteria for significance.

RESULTS

Extraction

The yield of CME and CMA extract obtained was 9.5 % w/w, & 11 % w/w respectively. The extract showed the presence of phytosterols, saponins, proteins, alkaloids, tannins, glycosides and flavonoids.

Nitrite Assay on Isolated Peritoneal Macrophages

The nitrite level (nitric oxide) produced in cell culture supernatants was measured at 24 hrs of treatment, showing that CMA extracts induced nitrite production in statistically significant P value. (*P<0.05*) at 832µg/ml (SI 2.080),,416µg/ml (SI 1.345), 208µg/ml (SI 1.384), 104µg/ml (SI 1.366), 52µg/ml (SI 1.377), 26 µg/ml (SI 1.462), 13µg/ml (SI 1.389), 6µg/ml (SI 1.364)..PHA showed significant stimulation (*p*<0.05)(SI 2.232).(Fig 1), and CME extract induced nitrite production in statistically significant P value, (*P<0.05*) at 832µg/ml (SI 1.829), 416µg/ml (SI 1.749), 208µg/ml (SI 1.561) 104µg/ml (SI 1.429), 52µg/ml (SI 1.401), 26µg/ml (SI 1.299), 13µg/ml (SI 1.144) concentrations than control. PHA (positive control) also showed a significant increase (*P<0.05*) in nitrite release (SI 2.23) (Fig 2).

In vitro Phagocytic Assay on NBT Dye Reduction

The *in vitro* phagocytic effects of different concentrations of CMA extract on the reduction of NBT dye on macrophages are presented in Fig. 1. The effect of CMA extract showed significant stimulation (*P<0.05*) on NBT reduction at 832µg/ml (SI 1.588) , 416µg/ml (SI 1.350), 208µg/ml (SI 1.367), 104µg/ml (SI 1.225), 52µg/ml (SI 1.315) 26 µg/ml (SI 1.187) & 13 µg/ml (SI 1.226) and CME extract showed significant stimulation (*P<0.05*) at 832µg/ml (SI 1.579) , 416µg/ml (SI 1.318), 208µg/ml (SI 1.193), 104µg/ml (SI 1.164), 52µg/ml (SI 1.151)

& 26 µg/ml (SI 1.145)13µg/ml (SI 1.074).PHA showed significant stimulation (*P*<0.05) of NBT reduction (S.I. 1.910).

In Vitro Assay on Cellular Lysosomal Enzyme Activity

In case of lysosomal enzyme activity CMA extract (fig 1) showed at 832µg/ml (SI 1.606), 416µg/ml (SI 1.281), 208µg/ml (SI 1.157), 26 µg/ml (SI 1.154), and in CME extract (fig 2) showed significant stimulation (*P<0.05*) at 832µg/ml (SI 1.423), 416µg/ml (SI 1.203) & 13 µg/ml (SI 1.277). PHA showed significant stimulation (*P*<0.05) of lysosomal enzyme release (SI 1.937) for both extracts.

Myeloperoxidase Activity Assay

The effect of CMA & CME extract on myeloperoxidase activity of macrophages is presented in Fig. 1 & Fig 2 respectively. The CMA extract showed significant (*P*<0.05) stimulation of myeloperoxidase activity of macrophages at 832µg/ml (SI 1.656), 416µg/ml (SI 1.320), 208µg/ml (SI 1.192), 52µg/ml (SI 1.132), 26 µg/ml (SI 1.188), 13µg/ml(SI 1.129), 6.5 µg/ml (SI 1.135) & in case of CME extract at 832µg/ml (SI 1.448), 416µg/ml (SI 1.279), 208µg/ml (SI 1.206) 104µg/ml (SI 1.105), as compared to control wells. Positive control, PHA showed significant stimulation with SI value (1.862).

TABLE 1

In-vitro effect of CMA on release of nitric oxide,NBT reduction, Lysosomal and Myeloperoxidase activity of isolated macrophages

Sr No.	Concentration (µg/ml)	Stimulation Index(SI)			
		NO	NBT	Lysosomal	MPO
1	Control	1.000 ±0.02	1.000 ±0.01	1.000 ±0.03	1.000 ±0.01
2	CMA(832)	2.080 ±0.06	1.588 ±0.02	1.606 ±0.02	1.656 ±0.04
3	CMA(416)	1.345 ±0.03	1.350 ±0.02	1.281 ±0.03	1.320 ±0.04
4	CMA(208)	1.384 ±0.03	1.367 ±0.01	1.157 ±0.03	1.192 ±0.02
5	CMA(104)	1.366 ±0.01	1.225 ±0.03	1.054 ±0.04	1.085 ±0.02
6	CMA(52)	1.377 ±0.02	1.315 ±0.04	1.097 ±.0.01	1.132 ±0.03
7	CMA(26)	1.462 ±0.03	1.187 ±0.03	1.153 ±0.03	1.188 ±0.03
8	CMA(13)	1.389 ±0.03	1.226 ±0.02	1.096 ±0.04	1.129 ±0.01
9	CMA(6.5)	1.364 ±0.01	1.023 ±0.01	1.102 ±0.03	1.135 ±0.02
10	PHA(10)	2.232 ±0.01	1.910 ±0.02	1.937 ±0.06	1.862 ±0.02

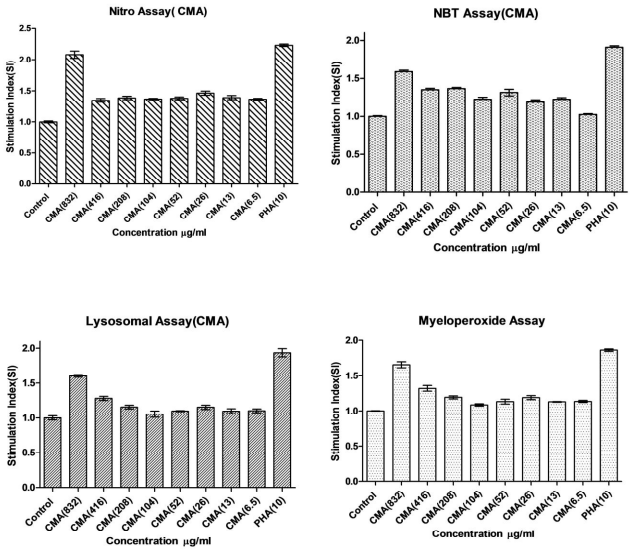

Figure 1

Table 2
In-vitro effect of CME on release of nitric oxide,NBT reduction, Lysosomal and Myeloperoxidase activity of isolated macrophages

Sr No.		Stimulation Index			
	Concentration (ug/ml)	NO	NBT	Lysosomal	MPO
1	Control	1.000±0.01	1.000±0.01	1.000±0.02	1.000±0.02
2	CME(832)	1.829±0.02	1.579±0.02	1.423±0.04	1.448±0.04
3	CME(416)	1.749±0.02	1.318±0.02	1.203±0.01	1.279±0.02
4	CME(208)	1.561±0.03	1.193±0.01	1.129±0.05	1.206±0.04
5	CME(104)	1.429±0.02	1.164±0.02	1.074±0.02	1.105±0.03
6	CME(52)	1.401±0.02	1.151±0.02	1.174±0.03	1.003±0.02
7	CME(26)	1.299±0.01	1.145±0.02	1.158±0.04	1.032±0.04
8	CME(13)	1.144±0.03	1.074±0.01	1.277±0.14	1.035±0.01
9	CME(6.5)	0.969±0.02	1.000±0.01	1.155±0.04	1.057±0.04
10	PHA(10)	2.231±0.02	1.910±0.01	1.937±0.05	1.862±0.05

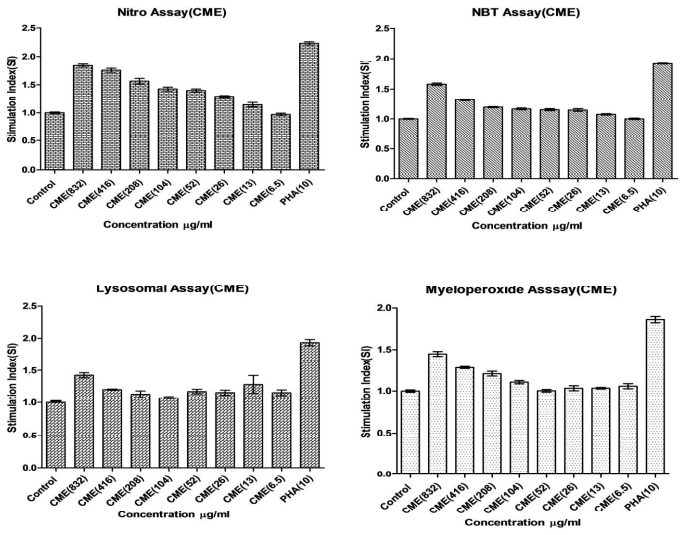

Figure 2

DISCUSSION

Immunomodulation through stimulation or suppression may help in maintaining a disease-free state. Agents that activate host defense mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy (Wagner H. 1984). There is a growing interest in identifying herbal immunomodulators ever since their possible use in modern medicine has been suggested (Tiwari U, Rastogi B, 2004). The main objective of the study was to investigate the immunomodulatory effects of aqueous and ethanolic extract of *Capparis moonii* .Macrophages have been known to play an important role in the host protection against a wide range of tumors and microorganisms. Macrophages also presents antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. When activated, macrophages increase the phagocytic activity and release various materials such as cytokines and reactive intermediates and then carry out non-specific immune responses. There has been great interest in reactive nitrogen intermediates, nitric oxide (NO), because of its antibacterial and

antitumor effect (Lowenstein C.J, Snyder S.H ,1992). NO mediates diverse functions, including vasodilatation, neurotransmission and inflammation (Aurasorn S, Kornkanok I, 2008). A very high NO production indicates increased phagocytosis and bactericidal activity, which is supported by the data, presented in Fig. 1. Macrophages play an important role in defense mechanism against host infection and in killing tumour cells. Higher reduction of NBT dye by CMA & CME extract represents a higher activity of oxidase enzyme, reflecting stimulation of phagocytes in proportion to intracellular killing. For lysosomal enzyme activity, the transformation of *p*-NPP to coloured compound by the acid phosphatase of the stimulated macrophages correlates to the extent of degranulation in phagocytosis (Benencia F, Courreges MC,1999). Phagocytosis of particles by macrophages is usually accompanied by a burst of oxidative metabolism allowing the generation of reactive oxygen species which can be detected through an assay based on the reduction of NBT (Brown KE, Brunt EM, 2001) The effect of various concentrations of CMA & CME extract on the reduction of NBT dye and lysosomal enzyme activity response of macrophages were studied for phagocytic assay. CMA & CME extract appeared to produce phagocytic stimulation with dose response relationship in lysosomal enzyme activity evaluation.

Myeloperoxidase, a heme protein secreted by neutrophils and macrophages, which uses the oxidizing potential of $H_2O_2$to convert chloride ion into hypochlorous acid (HOCl). A potent bactericidal agent, HOCl is a critical component of host defenses against invading bacteria, fungi, and viruses (Xie O.W, Cho H.J.,) The increase in the stimulation index of myeloperoxidase by the exposure of CMA & CME extract indicates enhanced defense capability of these cells to pathogenic organisms. The release of immune mediators from murine peritoneal macrophages was significantly stimulated by the exposure of the extract. Murine isolated peritoneal macrophages incubated with the CMA & CME extract at different concentrations ranging between 832 – 6.5 µg/ml for 24h, showed a significant activation of macrophages by modulating the secretion of various mediators including nitric oxide (NO), lysosomal enzyme and myeloperoxidase activity. This suggests that CMA & CME extract can effectively strengthen innate immunity against foreign particles (Miller LE. 1991).The present investigation suggests that *Capparis moonii aqueous and ethanolic extracts* had stimulated the phagocytic index in murine peritoneal macrophages.

CONCLUSION

The studies have demonstrated immunostimulating properties of both the ethanol & aqueous extract of Capparis *moonii* fruits in various *in vitro* experimental methods. Further studies to elucidate the exact immunostimulatory mechanism of *Capparis moonii* need to be explored. In-vivo studies also need to be done to correlate with the in-vitro results.

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REFERENCES

1. Agarwal R, Diwanay S, Patki P, Patwardhan B., Studies on immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation. Journal of Ethnopharmacology, 67, 1999, 27–35.
2. Aurasorn S, Kornkanok I, Pattana S., Effect of Bacopa monniera Linn. Extract on

Murine Immune Response In Vitro; Phytotherapy Research; 22, 2008, 1330 – 1335.
3. Benencia F, Courreges MC, Coulombie FC, Effect on the phagocytic activity and respiratory burst response of peritoneal macrophages; Immunopharmacology, 41, 1999, 45–53.
4. Brown KE, Brunt EM, Heinecke JW, Immunohistochemical detection of myeloperoxidase and its oxidation products in kupffer cells of human liver, Am. J. Pathol., 159, 2001, 2081-2088.
5. Choi C. Y, Kim J. Y., Kim Y. S. , Chung Y. C. Hahm K. S., Jeong H. G. Augmentation of macrophage functions by an aqueous abstract isolated from Platycodon grandiflorum, Cancer letter 166, 2001, 17-25
6. Choi E . M ., Hwang J.K. Effects of methanolic extract and fractions from Litsea cubeba bark on the production of inflammatory mediators in RAW264.7 cells, fitoterapia, 75, 2004, 141-148)
7. Dahanukar S. A., Thatte U.M. 1997, current status of Ayurveda in Phytomedicine ,4,359
8. Gottlieb A.A., Gottlieb M.S. Scholes V.E. 1987, Reconstitution of immune functions in AIDS /ARC. Concepts in Immunopathology 4, 261-274.
9. Lakshmi B. S. Kanaujia A, Duggar R, Pannakal ST, Yadav SS, Katiyar CK, Bansal V, Anand S, Sujatha S, : Insulinomimetic activity of two new gallotannins from the fruits of Capparis moonii. Biorg.Med.Chem.2010 ,18;11;3940-5
10. Lee S., Suk K. Heme oxygenase-1 mediates cytoprtective effects of immunostimulation in microglia; Biochemical Pharmacology 74; 723-729).
11. Lowenstein C.J, Snyder .H., Nitric oxide, a novel biologic messenger. Cell 70, 1992, 705.
12. Manosroi A, Saraphanchotiwitthaya A, Manosroi J., Effects of Pouteria Cambodiana extracts on invitro immunomodulatory activity of mouse immune system ; Fitoterapia 77, 2006, 189-193)
13. Miller L. E. (1991) In: Ludke, H.R., Peacock, J.E., Tomar, R.H. (Eds.), Manual of Laboratory Immunology, Lea and Febiger, London; 1–18.
14. P Rajesh , P selvamani, S latha, A saraswathi, V Rajesh Kannan , A review on chemical and medico biological applications of capparidaceae family
15. P Sunita, S Jha , S P Pattanayak: *In-vivo* Antitussive Activity of *Rudanti*. using Cough Model in Rodents
16. Prevention of carbon – tetra-chloride induced hepatoxicity by the ethanolic extracts of Capparis moonii in rats .PHARMA.BIOLOGY,42;286(2004)
17. Salman H, Bergman M, Bessler H, Fenig E, Weiss J, Beilin B, Djaldetti M., Decreased phagocytic capacity of rat peritoneal macrophages following photon abdominal irradiation, Cancer Letters, 147 (1–2), 1999, 175–179
18. Sharma P. 1983 Charak Samhita. Chikitasasthana. Chaukhamba Orientalia, Varanasi , India . P. 54
19. Son E.H, Moon EY, Rhee D.K.,, Pyo S., Stimulation of various functions in murine peritoneal macrophages by high mannuronic acid-containing alginate (HMA) exposure in vivo., International Immunopharmacology, 1, 2001, 147–154.
20. Stanilova S.A, Zhelev Z.D., Dobreva ZG., Preliminary studies on the immunomodulatory effect of the C3 binding glycoprotein isolated from Cuscuta europea; International Journal of Immunopharmacology, 22, 2000, 15-24.
21. Thatte U. M., Dahanukar S.A.,1986, Ayurveda and contemporary scientific thought , trends in pharmacological sciences 7, 247
22. Tiwari U, Rastogi B, Singh P, Saraf DK, Vyas SP., Immunomodulatory effects of aqueous extract of Tridax procumbens in experimental animals; Journal of Ethnopharmacology, 92, 2004, 113–119.

23. Trease, G.E., Evans , M. C. , 1983 , Text book of Pharmacognosy, 12th edition , Balliere, Tindall, London pp 343 -383.
24. Wagner H. 1999 In: Dhanukar S. A., Thatte U. M., Rege N. M., Birkhauser (Eds.), Immunomodulatory agents from plants, Verlag, Basel. Switzerland, pp. 289-323.
25. Wagner H. Immunostimulatory drugs of fungi and higher plants. In: Hiroshi Hikino, N.R., Farnsworth (Eds.), Economic and Medicinal Plant Research,vol. I. Academic Press, London, 1984, 113–153.
26. Xie O.W, Cho H.J., Calaycay J, Mumford RA, Swiderek KM, Lee TD, Ding A, Troso T, Nathan ., Cloning and characterization of inducible nitric oxide synthase from mouse macrophages ; Science1992,256,225