Immunomodulatory Effects of *Triphala* and its Individual Constituents: A Review

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The role of plant extracts and Ayurvedic polyherbal preparations in treating various ailments has been acknowledged since time immemorial. Studies based on the effect of these extracts in treatment of different diseases have also been well documented. Indian medicinal literature also emphasizes the synergistic effect of polyherbal drugs in restoring and rejuvenating immune system. This review focuses on the immunomodulatory potential of the polyherbal preparation, *Triphala* and its three constituents, *Terminalia bellerica*, *Terminalia chebula* and *Emblica officinalis*. The role of *Triphala* and its extract has been emphasized in stimulating neutrophil function. Under stress condition such as noise, *Triphala* significantly prevents elevation of IL-4 levels as well as corrects decreased IL-2 and IFN-γ levels. Under the condition of inflammatory stress its immunosuppressive activity is attributed to its inhibitory action on complement system, humoral immunity, cell mediated immunity and mitogen-induced T-lymphocyte proliferation. The aqueous and alcoholic extracts of the individual constituents reportedly enhance especially the macrophage activation due to their free radical scavenging activity and the ability to neutralize reactive oxygen species. This study thus concludes the use of *Triphala* and its three individual constituents as potential immunostimulants and/or immunosuppressants further suggests them to be a better alternative for allopathic immunomodulators.

Key words: *Triphala, Terminalia bellerica, Terminalia chebula, Emblica officinalis,* Immunomodulator

Ayurveda, the oldest healing science, focuses on treating different ailments through balancing the three pillars of life, *vat, pitta* and *kaf*. The role of different plant extracts in maintaining this balance and also treating various diseases is also well documented. However, in recent past in-depth research is focusing more on the phytochemical analysis of these extracts and their effects on various disease conditions *in vitro*. These plant extracts have proven to be important for reestablishing body's equilibrium and providing resistance against infection. They also possess the restorative and rejuvenating powers as they act on the immune system and positively affect the response of the body towards infection[1].

Immunomodulation is the process that alters the immune system of the host resulting in either immunostimulation or immunosuppression thus regulating or normalizing it. Hence, immunomodulators referred to as biological response modifiers, improve the host defense mechanism against diseases by striking a balance between regulatory and effector cells[2,3]. Using this quality of biological mediators, various alternative Ayurvedic formulations have been developed for various diseases where they either activate the host defense mechanism e.g. in case of impaired immune response or can selectively suppress it in conditions like autoimmune disorders and hypersensitivity. Such immunomodulatory properties of various medicinal plants provide an alternative to conventional synthetic drug therapy, which causes side effects, allergic reactions, tolerance to drugs and increased resistance of microorganisms to antibiotics.

The active components of various medicinal plants regulate the immune system by interacting with various immunocytes and regulating their effector mechanism for instance, cytokines and their receptors. These active components, referred to as *rasayanas* in Ayurveda, in sync with balanced diet impart vigor and longevity to individuals. This is called as *rasayana* therapy. Apart from this these active components play crucial role in enhancing body's resistance

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towards various diseases, memory and energy, which ultimately balances the health of the individual as a whole.

One of the important *rasayanas* in Ayurveda is *Triphala*, a polyherbal *tridoshic rasayana* consisting of *Terminalia bellerica Roxb.* (Family Combretaceae), *Terminalia chebula Retz*. (Family Combretaceae) and *Emblica officinalis* (Family Euphorbiaceae) in equal proportions. The present article aims at highlighting the immunomodulatory properties of the Ayurvedic *rasayana Triphala* and its three different constituents. It further focuses on generating awareness and acceptance of such Ayurvedic formulations in the society.

TERMINALIA CHEBULA RETZ.

Geographical distribution, phytochemistry and therapeutic uses:

T. chebula Retz. belongs to family Combretaceae and is found abundantly in different states of India like Madhya Pradesh, Gujarat, Maharashtra, Tamil Nadu, Karnataka and Bengal[4]. It is commonly called as Chebulic Myrobalan in English, *Harad* or *Harra* in Hindi and *Abhaya* in Sanskrit[5-7]. The tree is deciduous whose fruits are drupes with longitudinal wrinkles and a fibrous pericarp. They are oval to ellipsoid, 2-4 cm long and 1.5-2.5 cm broad. They are reported to contain tannins (30-40%) e.g. chebulinic acid[8], neochebulinic acid, corilagin, chebulagic acid[9,10], gallic acid, ellagic acid, punicalagin, terchebin and terflavin A. They also have flavonoids e.g. luteolin, rutins and quercetin in them[11]. Apart from these, they also contain other phytochemicals such as anthraquinones, saponins, β-D-glucogallin, 1,3,6-trigalloyl glucose, 1,2,3,4,6-penta-O-galloyl and various other carbohydrates, amino acids and fatty acids[12-17].

Due to presence of range of biologically active compounds, the fruits of *T. chebula* have been used in traditional medicine to combat a number of ailments of upper respiratory tract, gastrointestinal tract, urinary tract and skin[15,18-24]. These active compounds are effective in treating cancer[15] and other diseases of the heart, nervous system, bones and joints. Reportedly, the active components of the fruit have an antiageing effect, thus increasing the life span of the individual[11].

It has been reported to be an effective antibacterial agent against a wide range of Gram-positive and Gram-negative bacteria[25-28], antifungal agent against various pathogenic fungi[28-31] and an antiviral agent against swine influenza A virus, HSV-1, HIV-1 and cytomegalovirus[32-34]. The studies conducted have also inferred that the fruits of *T. chebula* act strongly against HBS antigen, inhibit HBV DNA polymerase and also significantly increased IFN-γ and IL-2 levels in peripheral blood mononuclear cell culture, thus emphasizing its antiHBV activity[35]. Further the studies have indicated it to be used as an antioxidant[36,37], antiinflammatory, antianaphylactic, antimutagenic[38,39], antinociceptive[40] and in wound healing activities[41,42].

Immunomodulatory activity:

The initial study on aqueous extract of *T. chebula* for its immunomodulatory activities has been reported. The study was based on assessment of humoral antibody titre and delayed type hypersensitivity (DTH) test[43]. A detailed study on immunomodulatory activity of its aqueous extract has also been reported, where the model animals were pretreated with 500 mg/kg of extract orally and challenged with 50 000 CFU of *S. typhimurium*. The animals showed $3\times10^3/mm^3$ increase in WBC count and 4% increase in lymphocyte count as compared to saline treated control animals. It was also reported that there was 102% increase in lymphocyte proliferation and 28.87% increase in foot pad thickness as compared to the infected control in DTH test. Thus the study concluded that the extract shows its protective effect through its immunomodulatory activity in mice against typhoid[44]. The aqueous extract reportedly increased humoral antibody titer[43] and therefore, it can be concluded that killing of *S. typhimurium* takes place via humoral response too.

The biologically active compounds such as chebulagic acid, gallic acid and ellagic acid make *T. chebula* highly potent antioxidant, which may be responsible for its immunomodulatory activity[24,45,46]. Its extract neutralizes reactive oxygen species (ROS) and scavenges free radicals. The free radicals are responsible for causing inflammation by stimulating release of cytokines such as IL-1, TNF-α and IFN-β, which stimulate additional neutrophils and macrophages at site of inflammation[47]. Thus, different antioxidants of the extract exhibit immunosuppressive

properties, which help in neutralizing these important inflammatory mediators.

Another study was conducted on the alcoholic extract of *T. chebula* focusing on its immunomodulatory activity[48]. The results indicated elevated levels of different antioxidant enzymes, glutathione and T- and B-cells suggesting its role in immunostimulation. Further the study reported increase in concentration of melatonin in pineal glands as well as the cytokines such as IL-2, IL-10 and TNF-α which play crucial role in immunity, thereby focusing on its immunostimulant property.

TERMINALIA BELLERICA ROXB.

Geographical distribution, phytochemistry and therapeutic uses:

T. bellerica Roxb. also belongs to family Combretaceae and apart from dry and desert regions of India, it occurs commonly in mixed deciduous forests of Punjab, Haryana, Uttar Pradesh, Madhya Pradesh and Maharashtra[5,7,16,49,50]. It has various names i.e. Beleric Myrobalan in English, *Baheda* in Hindi and *Bibhitaki* in Sanskrit[51]. The fruits of *T. bellerica* Roxb. are ovoid drupes, 2-3 cm long and 1-2 cm in diameter, grey to dark brown in colour, 5 distinct ridges present externally, densely covered with hair and velvet to touch. Fruits narrow into a very short stalk which on drying leaves a very prominent scar at the base[6,52,53]. The fruits are reported to contain gallotannic acid, ellagic acid, gallic acid, lignins such as termilignin and thannilignin and anolignan-B[54], 7-hydroxy-3, 4-flavone, ethyl gallate, galloyl glucose, chebulaginic acid, phenyllemblin, β-sitosterol, anthraquinones, glycosides such as bellaricanin[54,55] and other carbohydrates[51,54,56]. The oil content of the fruit is high (30-40%) as it contains palmitic, stearic, oleic and linoleic acid[15,16]. The fruit extract is reported to be acrid, astringent, antioxidant, antipyretic, antiemetic, antiinflammatory, anthelmintic, antidiabetic, antidiarrhoeal and analgesic[57-62]. It is also a good brain tonic, expectorant and laxative; used as a blood purifier and a rejuvenating drug[63-65]. The fruit extract possesses high antimicrobial activity specifically against Gram-positive bacteria[28,66] e.g. *B. subtilis* and *S. aureus*, pathogenic bacteria and yeasts and moulds e.g. *C. albicans* and *A. niger*[28]. However, it is shown to decrease pathogenicity of Gram-negative motile bacteria e.g. *S. typhi, S. typhimurium, E. coli* and *P. aeruginosa* by rendering them less motile[66].

Immunomodulatory activity:

Lately, the focus of the work is on assessing the immunomodulatory effect of *T. bellerica* extract. Since different activities of the extract have been attributed to the various biologically active compounds present in it, it is likely that some of these influence the host immune system as well. Gallic acid of many different plant extracts has been reportedly responsible for increasing ROS production in macrophages resulting in their increased phagocytic activity[67-69]. *T. bellerica* fruit extract is also rich in gallic acid content and therefore has been reported to be responsible for increasing the phagocytic activity of macrophages. The other mechanism for the enhanced phagocytic activity of the extract is due to some alteration in the mechanism of action of related enzyme such as phosphotyrosine phosphatase resulting in superoxide anion production[70]. This property of the extract makes it a potential drug against microbial infection and cancer[71]. Another study performed on mouse models reported the methanol extract of *T. bellerica* to be a potent stimulus for enhanced T-lymphocyte proliferation as compared to phytohaemagluttinin (PHA) alone. When the extract was administered with lipopolysaccharides (LPS) and poke weed mitogen (PWM), T-cell-independent B-cell proliferation was more enhanced indicating better cell-mediated immunity (CMI) than humoral-mediated immunity (HMI) and also reported to induce mouse spleenic B-cell through T-cell independent mechanism. Therefore, the study concluded that gallic acid from different plant extracts is the active component responsible for stimulation of immune system of mice[72]. These results were later confirmed by another study[73].

EMBLICA OFFICINALIS LINN.

Geographical distribution, phytochemistry and therapeutic uses:

Emblica officinalis Linn. (Family Euphorbiaceae) is indigenous to mixed deciduous forests of tropical India. In English it is called as Embelic Myrobalan or Indian Gooseberry, in Hindi it is called as *Amla* and in Sanskrit it is mentioned as *Amlaki* or *Amlakan*[5]. Fruits are pale green, globose, fleshy and 6-lobed with 3 segments. They are 1.5-2.5 cm in diameter and its mesocarp is edible and the endocarp is stony[49,52,74].

On ripening they are extremely acidic, astringent with distinct bitter flavor. They are also cooling and refrigerant in nature[75].

It is reported to contain phenolic constituents like gallic acid and its derivatives, mucic acid and its derivatives, corillagin, chebulagic acid, putrajivain A[76,77]. They possess high amounts of tannins like emblicanin A and B, punigluconin and pedunculagin[78], flavonoids like quercetin[79,80] and alkaloids like phyllantin and phyllantidin[81]. Various groups have reported high amounts of vitamin C[82-86] and considerably high amounts of minerals, proteins and amino acids like proline, alanine, cysteine, glutamic acid, aspartic acid and lysine. The fruits also contain glucose, fibers, phosphorus, iron and calcium[12,87].

The fruit is highly beneficial as cytoprotective[88], hepatoprotective[89,90], radioprotective[91], gastroprotective[92] and antitussive agent[93]. It is used to treat ophthalmic disorders, diarrhea, diabetes, scurvy, tumor, and ulcer[92,94-102] and protects against hyperthyroidism[103], cataract[100], ischemic reperfusion induced oxidative stress[104,105], atherosclerosis and hyperlipidemia[106-109]. It is also a potent antibacterial agent against Gram-positive and Gram-negative bacteria[28,110-112] as well as an antifungal agent[28,111].

Immunomodulatory activity:

Various studies have proved the fruit extract to be strongly immunomodulatory when Cr (VI) was used as immunosuppressant drug. It possesses antiapoptotic property and ceases DNA fragmentation, thus countering the immunosuppressive effect of Cr (VI) on lymphocyte proliferation. Further, it considerably restores IL-2 and IFN-γ production. This helped in restoring antioxidant status against Cr (VI) induced free radical production back to control level[113,114].

In another study, the immunomodulatory activity of aqueous extract of *E. officinalis* was reported. It showed that *E. officinalis*-treated mice had significantly higher antisheep RBC titer and DTH reaction compared to the control. This was concluded because of significant increase in WBC count and % lymphocyte distribution in *E. officinalis*-treated mice, suggesting its ability to stimulate haemolymphopoetic system[115]. *E. officinalis*-treated groups also produced high serum protein especially serum globulin and the mice also showed increase in spleen weight suggesting increased immunocompetence in them[116]. All these results indicated stimulant effect of *E. officinalis* on both CMI and HMI responses. Further the studies concluded that the extract caused significant increase in migration area as well as nitro blue tetrazolium (NBT) reduction of peritoneal macrophages in *E. officinalis*-treated mice as compared to control group indicating the role of extract in macrophage activation. This was accompanied by burst of oxidative metabolism generating ROS detected through NBT assay, confirmed the intracellular killing property of phagocytosing macrophages[115].

TRIPHALA

Triphala as mentioned earlier is made of equal amounts of dried fruits of *T. chebula, T. bellerica* and *E. officinalis*. Thus the phytochemicals present in it are those of its individual components, making it rich in gallic acid, tannins, chebulagic acid, ellagic acid, phenols and glycosides[117].

In Ayurvedic literature, *Triphala* is mentioned as a bowel regulator, tonic, cleanser, blood purifier and as an eyewash to counter many eye ailments e.g. conjunctivitis and to remove redness and soreness of eyes. It is also used to alleviate headache, dyspepsia, constipation, liver conditions and leucorrhoea. It is reported to be an effective antibacterial agent against Gram-positive and Gram-negative bacteria, antifungal agent[28], antidiabetic[68], radioprotective[118], antimutagenic[39], antioxidant[119,120], chemopreventive agent[121-123], analgesic, antipyretic[124-126], anticancer[15,123,127] and hypolipidaemic agent[128]. It is also reported to work against dental caries[129].

Immunomodulatory activity:

Triphala as a whole has been reported to be good for health and is used for its therapeutic actions. Recent extensive research has regarded it to be an extraordinary preparation with huge benefits such as adaptogenic and immunomodulation. A study was conducted to assay the immunomodulatory activity of *Triphala* using neutrophil functions like adherence, phagocytosis and NBT reduction in albino rats[130]. The study group of rats was exposed to noise stress of 100dB for 4 h/day for 15 days and their neutrophil function tests and corticosterone levels were assessed. The group that

was administered *Triphala* 1 g/kg/day for 48 days showed significantly enhanced avidity index with no change in neutrophil function and steroid levels. The *Triphala* administered group, immunized with sheep RBC (5×10^9 cells/ml), showed significant decrease in corticosterone levels. The groups viz. noise stress and noise stress immunized groups showed significantly suppressed neutrophil function, followed by significant increase in corticosterone levels. The study showed that administration of *Triphala* in both groups i.e. noise stress alone and noise stress immunized group greatly prevented the effect of noise stress, which was evident by its immunostimulant effect on neutrophil function and immunosuppressant effect on corticosterone levels of the model animals. Another study was conducted to evaluate the immunomodulatory effect of *Triphala* based on the analysis of the antibody titre, Pan-T, CD^{4+}/CD^{8+} lymphocyte phenotype in spleen and different cytokines like IL-2, IL-4 and IFN-γ[131]. Four groups of rats were employed namely, control, *Triphala* (1g/kg), noise stress (100dB for 4 h/day for 15 days), *Triphala*+noise stress immunized by sheep RBC (5×10^9 cells/ml). The results showed elevation in serum antibody titre and IL-4 levels accompanied by decreased IL-2, IFN-γ levels and reduction in Pan-T, CD^{4+}/CD^{8+}lymphocyte phenotype in spleen induced by noise stress. However, these effects were significantly prevented in the rats those were exposed to noise stress after being treated by *Triphala*, thus suggesting its therapeutic effectiveness.

Another study evaluated the immunomodulatory effects of *Triphala* powder on experimentally induced inflammation in mice[132]. The investigation was based on assessment of complement activity; HMI and CMI in mice and PHA-induced T-lymphocyte proliferation *in vitro*. The study was conducted on two groups of six mice each, both of which were inoculated with 0.1 ml of complete Freund's adjuvant in right hind paw to induce inflammation. The mice of both groups were orally administered with a dose of 500 mg/kg of *Triphala* powder, 1 h before induction of the adjuvant. The same dose continued for 5 days for one group while the other group was kept as control. *Triphala* administration showed significant inhibition of complement activity, HMI, CMI and mitogen-induced T-lymphocyte proliferation in a dose-dependent manner. These results suggested immunosuppressive activity of *Triphala* in experimentally-induced inflammation indicating its efficacy in treating inflammation and other autoimmune diseases.

The adaptogenic and immunomodulatory activity of *Triphala* megaext was assessed by a group of workers[133]. The megaext was prepared by mixing together six different extracts of *Triphala* made using non- polar to polar solvents (petroleum ether, benzene, chloroform, ethyl acetate, 70% ethanol and water). The extracts were then concentrated by distilling the solvent and air-dried. This megaext reportedly contained alkaloids, carbohydrates, glycosides, terpenoids, protein and amino acids, phenolics and tannins, flavonoids, oils, fats and saponins. The LD_{50} of this extract was determined to be 2000 mg/kg on Swiss albino mice. Five hundred and 1000 mg/kg doses were selected for *in vivo* study. The two parameters studied were carbon clearance assay (CCA) and DTH. The group of mice receiving 500 mg/kg p.o. as well as 1000 mg/kg p.o. showed significant increase in phagocytic index as compared to the control group, which received 25 mg/kg p.o. livomisole as a standard immunomodulator. This increase in carbon clearance index clearly shows enhancement of phagocytic function of mononuclear macrophages and thus non-specific immunity. The megaext thus enhanced the phagocytic function, which was exhibited by the clearance rate of carbon by the cells of reticulo-endothelial system.

In the same two groups DTH was used to study the CMI response of *Triphala* megaext where the foot pad edema was induced in Swiss albino mice and the paw edema value was observed. Increase in paw edema value was indicative of CMI response. There are two phases of DTH response. The first comprises of sensitization phase, which comes after the initial contact with sheep RBC antigen followed by the effector phase that comes after subsequent exposure to sheep RBC antigen. In this latter stage a variety of cytokines are secreted by T_H1 cells that help in recruitment and activation of macrophages and other nonspecific inflammatory mediators. Both the treated groups showed an increase in DTH as compared to the control groups. This clearly indicates the stimulatory effect of megaext on immune cells in response to T-cell-dependent antigen.

CONCLUSION

Use of traditional medicines for improving immunity and treating various diseases has been approved by WHO. India has a rich documented history of traditional medicines such as *Sushrut Samhita* and *Charak Samhita*. Presently for treating various ailments allopathic drugs are preferred, which are not only very expensive but pose a great threat by causing mild to severe side effects. None of these problems occur with the prescribed dosage of plant-based medicines.

To shift the focus from conventional allopathic drugs to traditional plant based drugs a more comprehensive and focused study is required targeting molecular level by isolating, identifying and conducting phase-wise clinical trials of active compounds. This would not only help in generating awareness and greater acceptance amongst physicians but also among general public.

In this regard, this review is a step towards evaluating the pharmacological properties of *Triphala* and its three different constituents. The review indicates presence of different active compounds in them such as gallic acid, chebulagic acid, ellagic acid, flavonoids, tannins and phenols, which are responsible for its effective immunostimulatory and immunosuppressant property making it a strong contender as a plant based Ayurvedic immunomodulator.

REFERENCES

1. Kumar UA, Manjunath C, Thaminzhmani T, Ravi Kiran Y, Brahmaiah Y. A review on immunomodulatory activity plants. Indian J Novel Drug Delivery 2012;4:93-103.
2. Sehar I, Kaul A, Bani S, Pal HC, Saxena AK. Immune up-regulatory respose of a non-caloric natural sweetener, stevioside. Chem Biol Interact 2008;173:115-21.
3. Agrawal SS, Khadase SC, Talele GS. Studies on immunomodulatory activity of *Capparis zeylanica* leaf extracts. Int J Pharm Sci Nanotechnol 2010;3:887-92.
4. Beusher N, Bodinet C, Neumann-Haefelin D, Marston A, Hostettmann K. Antiviral activity of African medicinal plants. J Ethnopharmacol 1994;42:101-9.
5. Chopra R, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. 1st ed. National Institute of Science Communication, New Delhi, India: CSIR; 1956. p. 242.
6. Chadha YR. The Wealth of India: Raw Materials. Vol. 10. New Delhi, India: CSIR; 1976. p. 285.
7. API, Govt. of India, Ministry of Health and Family Welfare. New Delhi, India: Dept. of ISM and H; 1986.
8. Lee HS, Koo YC, Suh HJ, Kim KY, Lee KW. Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation end product-induced endothelial cell dysfunction. J Ethnopharmacol 2010;131:567-74.
9. Reddy DB, Reddy TC, Jyotsna G, Sharan S, Priya N, Lakshmipathi V, *et al.* Chebulagic acid, a COX-LOX dual inhibitor isolated from the fruits of *Terminalia chebula* Retz., induces apoptosis in COLO-205 cell line. J Ethnopharmacol 2009;124:506-12.
10. Reddy TC, Aparoy P, Babu NK, Kumar KA, Kalangi SK, Reddanna P. Kinetics and docking studies of a COX-2 inhibitor isolated from *Terminalia bellerica* fruits. Protein Pept Lett 2010;17:1251-7.
11. SuryaPrakash DV, Satya NS, Avanigadda S, Vanglapati M. Pharmacological review on *Terminalia chebula*. Int J Res Pharma Biomed Sci 2012;3:679-83.
12. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. 1. CDRI, Lucknow, New Delhi, India: National Institute of Science Communication; 1990.
13. Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants. Vol. 3. CDRI, Lucknow, New Delhi, India: Publication and Information Directorate, CSIR; 1993.
14. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants. Vol. 5. CDRI, Lucknow. New Delhi, India: Publication and Information Directorate, CSIR; 1995. p. 841.
15. Saleem A, Husheem M, Harkonen P, Pihlaja K. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz fruit. J Ethnopharmacol 2002;81:327-36.
16. Gokhale SB, Kokate CK, Purohit AP. Pharmacognosy. 18th ed. Pune, India: Nirali Prakashan; 2003. p. 258-9.
17. Juang LJ, Sheu SJ. Chemical identification of the sources of commercial *Fructus chebulae*. Phytochem Anal 2005;16:246-51.
18. Phadke SA, Kulkarni SD. Screening of *in-vitro* antibacterial activity of *Terminalia chebula*, *Eclapta alba* and *Ocimum sanctum*. Indian J Med Sci 1989;43:113-7.
19. Hamada S, Kataoka T, Wo JT, Yamada A, Yoshida T, Nishimura T, *et al.* Immunosuppressive effects of gallic acid and chebulagic acid on CTL-mediated cytotoxicity. Biol Pharma Bull 1997;20:1017-9.
20. Tamhane MD, Thorat SP, Rege NN, Dahanukar SA. Effect of oral administration of *Terminalia chebula* on gastric emptying: An experimental study. J Postgrad Med 1997;43:12-3.
21. Shin TY, Jeong HJ, Kim DK, Kim SH, Lee JK, Kim DK, *et al.* Inhibitory action of water soluble fraction of *Terminalia chebula* on systemic and local anaphylaxis. J Ethnopharmacol 2001;74:133-40.
22. Ahn MJ, Kim CY, Lee JS, Kim TG, Kim SH, Lee CK, *et al.* Inhibition of HIV-I integrase by galloyl glucoses from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis*. Planta Med 2002;68:457-9.
23. Na M, Bac K, Kang SS, Mim BS, Yoo JK, Kamiryo Y, *et al.* Cytoprotective effect on oxidative stress and inhibitory effect on cellular aging of *Terminalia chebula* fruit. Phytother Res 2004;18:737-41.
24. Lee HS, Won NH, Kim KH, Lee H, Jun W, Lee KW. Antioxidant effects of aqueous extract of Terminalia chebula *in vivo* and *in vitro*. Biol Pharm Bull 2005;28:1639-44.
25. Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against *Helicobacter pylori*. Int J Antimicrob Agents 2001;18:85-8.
26. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts. J Nat Med 2008;62:259-62.
27. Kannan P, Ramadevi SR, Waheeta H. Antibacterial activity of *Terminalia chebula* fruit extract. Afr J Microbiol Res 2009;3:180-4.
28. Nagar S, Belapurkar P, Barua PT, Goyal P. Antimicrobial and phytochemical analysis of Triphala and comparison with its individual constituents. Natl J Life Sci 2011;8:101-3.
29. Dutta BK, Rahman I, Das TK. Antifungal activity of Indian plant extracts. Mycoses 1998;41:535-6.
30. Bajpai VK, Rahman A, Shukla S, Shukla S, Arafat SM, Hossain MA, *et al. In vitro* kinetics and antifungal activity of various extracts of *Terminalia chebula* seeds against plant pathogenic fungi. Arch Phytopathol Plant Prot 2010;43:801-9.

31. Shinde L, More SM, Junne SB, Wadje SS. The antifungal activity of five *Terminalia* species checked by paper disk method. Int J Pharma Res Dev 2011;3:36-40.
32. Ma H, Diao Y, Zhao D, Li K, Kang T. A new alternative to treat swine influenza A virus infection: Extracts from *Terminalia chebula* Retz. Afr J Microbiol Res 2010;4:497-9.
33. Yukawa TA, Kurokawa M, Sato H, Yoshida Y, Kageyama S, Hasegawa T, *et al.* Prophylactic treatment of cytomegalovirus infection with traditional herbs. Antiviral Res 1996;32:63-70.
34. Kim TG, Kang SY, Jung KK, Kang JH, Lee E, Han HM, *et al.* Antiviral activities of extracts isolated from *Terminalis chebula* Retz., *Sanguisorba officinalis* L., *Rubus coreanus* Miq. and *Rheum palmatum* L. against hepatitis B virus. Phytother Res 2001;15:718-20.
35. Mohan K, Paramasivam R, Chandran P, Veerasami V, Gnanasekaran A, Shanmugam A, *et al.* Inhibition of Hepatitis B virus DNA polymerase and modulation of TH1 and TH2 cytokine secretion by three Indian medicinal plants and its correlation with antiviral properties. J Pharm Res 2011;4:1044-6.
36. Suchalatha S, Srinivasulu C, Devi SC. Antioxidant activity of ethanolic extract of *Terminalia chebula* fruit against isoproterenol–induced oxidative stress in rats. Indian J Biochem Biophy 2005;42:246-9.
37. Chang CS, Lin CS. Development of antioxidant activity and pattern recognition of *Terminalia chebula* Retzius extracts and its fermented products. Hung Kuang J 2010;61:115-29.
38. Grover IS, Bala S. Antimutagenic activity of *Terminalia chebula* (myrobalan) in *Salmonella typhimurium*. Indian J Exp Biol 1992;30:339-41.
39. Kaur S, Arora S, Kaur K, Kumar S. The *in vitro* antimutagenic activity of Triphala an Indian herbal drug. Food Chem Toxicol 2002;40:527-34.
40. Kaur S, Jaggi RK. Antinociceptive activity of chronic administration of different extracts of *Terminalia bellerica* Roxb. and *Terminalia chebula* Retz. fruits. Indian J Exp Biol 2010;48:925-30.
41. Singh MP, Sharma CS. Wound healing activity of *Terminalia chebula* in experimentally induced diabetic rats. Int J Pharm Tech Res 2009;1:1267-70.
42. Choudhary GP. Wound healing activity of ethanolic extract of *Terminalia chebula* Retz. Int J Pharma Bio Sci 2011;2:48-52.
43. Shivaprasad HN, Kharya MD, Rana AC, Mohan S. Preliminary immunomodulatory activities of the aqueous extract of *Terminalia chebula*. Pharm Biol 2006;44:32-4.
44. Khan KH. Immunomodulatory activity of *Terminalia chebula* against *Salmonella typhimurium* in mice. Recent Res Sci Tech 2009;1:211-6.
45. Lee HS, Jung SH, Yun BS, Lee KW. Isolation of chebulic acid from *Terminalia chebula* Retz. and its antioxidant effect in isolated rat hepatocytes. Arch Toxicol 2007;81:211-8.
46. Tejesvi MV, Kini KR, Prakash HS, Subbiah V, Shetty HS. Antioxidant, antihypertensive, and antibacterial properties of endophytic *Pestalotiopsis* species from medicinal plants. Can J Microbiol 2008;54:769-80.
47. Conforti F, Sosa S, Marrelli M, Menichini F, Statti GA, Uzunov D, *et al.* The protective ability of Mediterranean dietary plants against the oxidative damage: The role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. Food Chem 2009;112:587-94.
48. Aher V, Wahi AK. Immunomodulatory activity of alcohol extract of *Terminalia chebula* Retz. combretaceae. Trop J Pharm Res 2011;10:567-75.
49. Bakhru HK. Herbs that heal: Natural Remedies for good health. 1st ed. New Delhi, India: Vision Books Pvt Ltd, Orient paperbacks; 1990. p. 39.
50. Chatterjee A, Pakrashi S. The treatise on Indian medicinal plant. Vol. 3. New Delhi, India: Publications and Information Directorate, CSIR; 1994. p. 76.
51. Saroya AS. Herbalism, Phytochemistry and Ethnopharmacology. India: Science Publisher; 2011. p. 357-61.
52. Singh V, Pande PC, Jain DK. A Text Book of Botany: Angiosperms. 1st ed. Meerut, India: Rastogi Publication; 2002.
53. Choudhary GP. Immunomodulatory activity of alcoholic extract of *Terminalia belerica* Linn. in mice. Der Pharmacia Lettre 2012;4:414-7.
54. API. Ayurvedic Pharmacopoiea of India. 1st ed. New Delhi, India: The Controller of Publications, Civil Lines; 2001. p. 34.
55. Singh A. Medicinal plants of the World. New Delhi, India: Oxford and IBH Co; 2006. p. 7.
56. IHP. Indian Herbal Pharmacopoeia. Revised ed. Mumbai: Indian Drug Manufacturer's Association; 2002. p. 429-38.
57. Tariq M, Hussain SJ, Asif M, Jahan M. Protective effect of fruit extracts of *Emblica officinalis* (Gaertn.) and *Terminalia belerica* (Roxb.) in experimental myocardial necrosis in rats. Indian J Exp Biol 1977;15:485-6.
58. Suthienkul O, Miyazaki O, Chulasiri M, Kositanont U, Oishi K. Retroviral reverse transcriptase inhibitory activity in Thai herbs and spices: Screening with Moloney murine leukemia viral enzyme. Southeast Asian J Trop Med Public Health 1993;24:751-5.
59. Shaila HP, Udupa AL, Udupa SL. Preventive actions of *Terminalia belerica* in experimentally induced atherosclerosis. Int J Cardiol 1995;49:101-6.
60. Anand KK, Singh B, Saxena AK, Chandan BK, Gupta VN, Bhardwaj V. 3,4,5,-Trihydroxy benzoic acid (gallic acid), the hepatoprotective principle in the fruits of *Terminalia belerica*-bioassay guided activity. Pharmacol Res 1997;36:315-21.
61. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998;62:183-93.
62. Aqil F, Khan MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Basic Microbiol 2005;45:106-14.
63. Nadkarni AK, Nadkarni KM. Indian Materia Medica. Vol. 1. Mumbai, India: Popular Prakashan; 1982. p. 1202.
64. Trease GD, Evans WC. Pharmacognosy. 15th ed. San Diego. California: Harcourt Brace and Co; 1997. p. 226 and 472.
65. Handa SS, Kapoor VK. Pharmacognosy. 2nd ed. New Delhi, India: Vallabh Prakashan, Educational publishers; 2002. p. 222-3.
66. Elizabeth KM. Antimicrobial activity of *Terminalia bellerica*. Indian J Clin Biochem 2005;20:150-3.
67. Tam PE, Hinsdill RD. Screening for immunomodulators: Effects of xenobiotics on macrophage chemiluminescence *in vitro*. Fundam Appl Toxicol 1990;14:542-53.
68. Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J Ethnopharmacol 2002;81:155-60.
69. Houde V, Grenier D, Chandad F. Protective effects of grape seed proanthocyanidins against oxidative stress induced by lipopolysaccharides of periodontopathogens. J Periodontol 2006;77:1317-9.
70. Carreras MC, Riobo NA, Pargament GA, Boveris A, Poderoso JJ. Effects of respiratory burst inhibitors on nitric oxide production by human neutrophils. Free Radic Res 1997;26:325-34.
71. Saraphanchotiwitthaya A, Sripalakit P, Ingkaninan K. Effects of *Terminalia bellerica* Roxb. methanolic extract on mouse immune response *in vitro*. Mj Int J Sci Tech 2008;2:400-7.
72. Hu ZQ, Toda M, Okubo S, Hara Y, Shimamura T. Mitogenic activity of (-) epigallocatechin gallate on B-cells and investigation of its structure-function relationship. Int J Immunopharmacol 1992;14:1399-407.
73. Kovacevic N, Colic M, Backovic A, Doslov-Kokorus Z. Immunomodulatory effects of the methanolic extract of *Epimedium alpinum in vitro*. Fitoterapia 2006;77:561-7.
74. Anonymous. The wealth of India: Raw materials. Vol. 3. New Delhi, India: Publications and Information Directorate, Council of Scientific and Industrial Research; 1952. p. 168-70.
75. Nadkarni KM, Nadkarni AK. Indian Materia Medica-with Ayurvedic, Unani-Tibbi, Siddha, Allopathic, Homeopathic, Naturopathic and Home Remedies. Mumbai, India: Popular Prakashan Private Ltd;

1999. p. 245 and 250.
76. Zhang YJ, Tanaka T, Yang CR, Kouno I. New phenolic constituents from the fruit juice of *Phyllanthus emblica*. Chem Pharm Bull (Tokyo) 2001;49:537-40.
77. Zhang YJ, Nagao T, Tanaka T, Yang CR, Okabe H, Kouno I. Antiproliferative activity of the main constituents from *Phyllanthus emblica*. Biol Pharma Bull 2004;27:251-5.
78. Ghosal S, Tripathi VK, Chauhan S. Active constituents of *Emblica officinalis*. Part I. The chemistry and antioxidant effects of two new hydrolysable tannins, emblicanin A and B. Indian J Chem 1996;35:941-8.
79. Gulati RK, Agarwal S, Agrawal SS. Hepatoprotective studies on *Phyllanthus emblica*. Linn. and quercetin. Indian J Exp Biol 1995;33:261-8.
80. Anila L, Vijayalakshmi NR. Flavonoids from *Emblica officinalis* and *Mangifera indica*- effectiveness for dyslipidemia. J Ethnopharmacol 2002;79:81-7.
81. Khanna P, Bansal R. Phyllantidine and phyllantine from Emblica officinalis Gaertn leaves, fruits, and *in vitro* tissue. Indian J Exp Biol 1975;13:82-3.
82. Scartezzini P, Speroni E. Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnopharmacol 2000;71:23-43.
83. Khopde SM, Priyadarsini KI, Mohan H, Gawandi VB, Satav JG, Yakhmi JV, *et al*. Characterizing the antioxidant activity of Amla (*Phyllanthus emblica*) extract. Curr Sci 2001;81:185-90.
84. Nisha P, Singhal RS, Pandit AB. Study on degradation kinetics of ascorbic acid in amla (*Phyllanthus emblica* L.) during cooking. Int J Food Sci Nutr 2004;55:415-22.
85. Paul DK, Shaha RK. Nutrients, vitamins and minerals content in common citrus fruits in the northern region of Bangladesh. Pak J Biol Sci 2004;7:238-42.
86. Scartezzini P, Antogoni F, Raggi MA, Poli F, Sabbioni C. Vitamin C content and antioxidant activity of the fruit and of the ayurvedic preparation of *Emblica officinalis* Gaertn. J Ethnopharmacol 2006;104:113-8.
87. Asolkar LV, Kakkar KK, Chakre OJ. Glossary of Indian medicinal plants with active principles. Part 2. New Delhi, India: Publication and Information Directorate, CSIR; 1992. p. 291-2.
88. Sai Ram M, Neetu D, Yogesh B, Anju B, Dipti P, Pauline T, *et al*. Cyto-protective and immunomodulating properties of Amla (*Emblica officinalis*) on lymphocytes: An *in vitro* study. J Ethnopharmacol 2002;81:5-10.
89. Jeena KJ, Joy KL, Kuttan R. Effect of *Emblica officinalis, Phyllanthus amarus* and *Picrorrhiza kurroa* on N-nitrosodiethylamine induced hepatocarcinogenesis. Cancer Lett 1999;136:11-6.
90. Jose JK, Kuttan R. Hepatoprotective activity of *Emblica officinalis* and chyavanprash. J Ethnopharmacol 2000;72:135-40.
91. Singh I, Sharma A, Nunia V, Goyal PK. Radioprotection of Swiss albino mice by *Emblica officinalis*. Phytother Res 2005;19:444-6.
92. Al-Rehaily AJ, Al-Howiriny TS, Al-Sohaibanib MO, Rafatullah S. Gastroprotective effects of 'Amla' *Emblica officinalis* on *in vivo* test models in rats. Phytomedicine 2002;9:515-22.
93. Nosal'ova G, Mokry J, Hassan KM. Antitussive activity of the fruit extract of *Emblica officinalis*. Phytomedicine 2003;10:583-9.
94. Ghosh A, Sharma A, Talukder G. Relative protection given by extract of *Phyllanthus emblica* fruit and an equivalent amount of vitamin C against a known clastogen-caesium chloride. Food Chem Toxicol 1992;30:865-9.
95. Xia Q, Xiao P, Wan L, Kong J. Ethnopharmacology of *Phyllanthus emblica* L. Zhongguo Zhong Yao Za Zhi 1997;22:515-74.
96. Bandyopadhyay SK, Pakrashi SC, Pakrashi A. The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention from indomethacin induced gastric ulcer. J Ethnopharmacol 2000;70:171-6.
97. Haque R, Bin-Hafeez B, Ahmad I, Parvez S, Panday S, Raisuddin S. Protective effects of *Emblica officinalis* Gaertn. in cyclophosphamide-treated mice. Hum Exp Toxicol 2001;20:643-50.
98. Hari Kumar KB, Sabu MC, Lima PS, Kuttan R. Modulation of haematopoetic system and antioxidant enzymes by *Emblica officinalis* Gaertn. and its protective role against gamma radiation induced damages in mice. J Radiat Res 2004;45:549-54.
99. Perianayagam JB, Sharma SK, Joseph A, Christina AJ. Evaluation of anti-pyretic and analgesic activity of *Emblica officinalis* Gaertn. J Ethnopharmacol 2004;95:83-5.
100. Suryanarayana P, Kumar PA, Saraswat M, Petrash JM, Reddy GB. Inhibition of aldose reductase by tannoid principles of *Emblica officinalis*: Implications for the prevention of sugar cataract. Mol Vis 2004;10:148-54.
101. Sancheti G, Jindal A, Kumari R, Goyal PK. Chemopreventive action of *Emblica officinalis* on skin carcinogenesis in mice. Asian Pac J Cancer Prev 2005;6:197-201.
102. Duan W, Yu Y, Zhang L. Antiatherogenic effects of *Phyllanthus emblica* associated with corilagin and it analogue. Yakugaku Zassi, 2005;125:587-91.
103. Panda S, Kar A. Fruit extract of *Emblica officinalis* ameliorates hyperthyroidism and hepatic lipid peroxidation in mice. Pharmazie 2003;58:753-5.
104. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosa S. Effect of bioactive tannoid principles of *Emblica officinalis* on ischemia-reperfusion-induced oxidative stress in rat heart. Phytomedicine 2002;9:171-4.
105. Rajak S, Banerjee SK, Sood S, Dinda KA, Gupta YK, Maulik SK. *Emblica officinalis* causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury in rats. Phytother Res 2004;18:54-60.
106. Mishra M, Pathak UN, Khan AB. *Emblica officinalis* Gaertn and serum cholesterol level in experimental rabbits. Br J Exp Pathol 1981;62:526-8.
107. Thakur CP, Thakur B, Singh S, Sinha PK, Sinha SK. The Ayurvedic medicines Haritaki, Amala and Bahira reduce cholesterol induced atherosclerosis in rabbits. Int J Cardiol 1988;21:167-75.
108. Mathur R, Sharma A, Dixit VP, Varma M. Hypolipidaemic effect of fruit juice of *Emblica officinalis* in cholesterol-fed rabbits. J Ethnopharmacol 1996;50:61-8.
109. Kim HJ, Yokozawa T, Kim HY, Tohda C, Rao TP, Juneja LR. Influence of amla (*Emblica officinalis* Gaertn.) on hypercholesterolemia and lipid peroxidation in cholesterol-fed rats. J Nutr Sci Vitaminol (Tokyo) 2005;51:413-8.
110. Javale P, Sabnis S. Antimicrobial properties and phytochemical analysis of *Emblica officinalis*. Asian J Exp Biol Sci 2010;91-5.
111. Aneja KR, Joshi R, Sharma C. *In vitro* antimicrobial activity of *Sapindus mukorossi* and *Emblica officinalis* against dental caries pathogens. Ethnobot Leaflets 2010;14:402-12.
112. Saradha Jyothi K, Subba Rao B. Screening of antibacterial activity of *Emblica officinalis* fruits. Pharmacologyonline 2011;3:848-52.
113. Sai Ram M, Neetu D, Deepti P, Vandana M, Ilavazhagan G, Kumar D, *et al.* Cytoprotective activity of Amla (*Emblica officinalis*) against chromium (VI) induced oxidative injury in murine macrophages. Phytother Res 2003;17:430-3.
114. Ganju L, Karan D, Chanda S, Srivastava KK, Sawhney RC, Selvamurthy W. Immunomodulatory effects of agents of plant origin. Biomed Pharmacother 2003;57:296-300.
115. Suja RS, Nair AM, Sujith S, Preethy J, Deepa AK. Evaluation of immunomodulatory potential of *Emblica officinalis* fruit pulp extract in mice. Indian J Anim Res 2009;113:103-6.
116. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 5th ed. San Diego, California: Academic Press; 1997. p. 932.
117. Naik GH, Priyadarsini KI, Hari M. Free radical scavenging reactions and phytochemical analysis of triphala, an ayurvedic formulation. Curr Sci 2006;90:1100-5.
118. Jagetia GC, Baliga MS, Malagi KJ, Kamath MS. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma radiation. Phytomedicine 2002;9:99-108.
119. Jagetia GC, Rao SK, Baliga MS, Babu KS. The evaluation of nitric oxide scavenging activity of certain herbal formulations *in vitro*: A preliminary study. Phytother Res 2004;18:561-5.
120. Naik GH, Priyadarsini KI, Bhagirathi RG, Mishra B, Mishra KP,

Banavalikar MM, *et al*. *In vitro* antioxidant studies and free radical reactions of triphala, an ayurvedic formulation and its constiuents. Phytother Res 2005;19:582-6.
121. Sandhya T, Mishra KP. Cytotoxic response of breast cancer cell lines, MCF7 and T47D to Triphala and its modification by antioxidants. Cancer Lett 2006;238:304-13.
122. Abraham S, Kumar MS, Sehgal PK, Nitish P, Jayakumar ND. Evaluation of inhibitory effect of Triphala on PMN- type matrix metalloproteinase (MMP-9). J Peridontol 2005;76:497-502.
123. Deep G, Dhiman M, Rao AR, Kale RK. Chemopreventive potential of Triphala (a composite Indian drug) on benzo(α) pyrene induced forestomach tumorigenesis in murine tumor model system. J Exp Clin Cancer Res 2005;24:555-63.
124. Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br J Pharmacol Chemother 1968;32:295-310.
125. Kasahara Y, Hikino H, Tsurufuji S, Watanabe M, Ohuchi K. Antiinflammatory actions of ephedrines in acute inflammations. Planta Med 1985;51:325-31.
126. Sabina EP, Rasool M. Analgesic, antipyretic and ulcerogenic effects of Indian Ayurvedic Herbal formulation Triphala. Res J Medicinal Plant 2007;1:54-9.
127. Kaur S, Michael H, Arora S, Härkonen PL, Kumar S. The *in vitro* cytotoxic and apoptotic activity of Triphala-an Indian herbal drug. J Ethanopharmacol 2005;97:15-20.
128. Saravanan S, Srikumar R, Manikandan S, Parthasarathy NJ, Devi RS. Hypolipidaemic effect of Triphala in experimentally induced hypercholesteremic rats. Yakugaku Zassi 2007;127:385-8.
129. Jagdish L, Anand Kumar VK, Kaviyarasan V. Effect of Triphala on dental bio-film. Indian J Sci Technol 2009;2:30-3.
130. Srikumar R, Parthasarathy NJ, Devi RS. Immunomodulatory activity of Triphala on neutrophil functions. Biol Pharm Bull 2005;28:1398-403.
131. Srikumar R, Parthasarathy NJ, Manikandan S, Muthuvel A, Rajamani R, Sheeladevi R. Immunomodulatory effect of Triphala during experimentally induced noise stress in albino rats. J Health Sci 2007;53:142-5.
132. Sabina EP, Rasool MK, Mathew L. *In vivo* and *in vitro* immunomodulatory effects of Indian Ayurvedic formulation Triphala on experimental induced inflammation. Pharmacologyonline 2009;2:840-9.
133. Sonkar R, Mishra RN. Immunomodulatory Activity of Triphala Megaext. Int J Res Pharm Biomed Sci 2011;2:575-8.

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