In vitro Antimicrobial and Antioxidant Activity of Immunity Booster Tea Formulation,

S. Farooq, *ZafarMehmood and Arunesh Kumar Dixit
The Himalaya Drug Company, Clemint Town, Dehradun, Uttarakhand, India
***Email: zafarmehmood31@gmail.com**
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Abstract-Currently, there is much growing interest in the use of medicinal plants as modulators of the complex immune system. Through a number of vast researches conducted in the area, it is being explored that many of the phytochemicals in the form of alkaloids,tannins, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties. Keeping in mind, the tremendous potential of the medicinal plants and their derived drugs, this study is undertaken and an immunobooster tea formulation (IMBF-01-20) was designed and *in vitro* antimicrobial and antioxidant activity was tested. The results showed strong antimicrobial activity of formulation(IMBF-01-20) against *Staph.aureus* with diameter of zone of inhibition of 32 mm in methanol extract followed by 20 mm in *E.coli.* While hexane extract also showing good inhibition of test organisms followed by Ethanol extract.Considering the growing demand of natural antioxidant, IMBF-01-20 was tested for their antioxidant activity using DPPH radical scavenging assay.Antioxidant activity (93.4%) was observed in immunobooster tea formulation and revealed it as a natural antioxidant.

Key words: Immunomodulators, Medicinal Plants, Immunobooster tea, Phytochemicals

Introduction

A broad range of health-care practices is required to exploit the beneficial effects of Ayurveda which is the most ancient 5000 years old system of medicines. Being the essence of Ayurvedic medicines, Indian medicinal plants manifest miraculous effects in curing a vast range of diseases and disorders among humans and can be better called as "elixirs of life." Currently, there is much growing interest in the use of these medicinal plants as modulators of the complex immune system. Through a number of vast researches conducted in the area, it is being explored that many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties.**(Preeti Sharma, et. al, 2017)**

Keeping in mind, the tremendous potential of the medicinal plants and their derived drugs, the present work done with a purpose to globally popularize the Indian herbal medicines as immunomodulators.

Concerted efforts have been made to explore the various biological and specific pharmacological activities and their active compounds all over the world. The antibacterial and antifungal activities of Indian medicinal plants are widely known against a variety of pathogenic and opportunistic microorganisms (Aqil and Ahmad, 2007). However, targeted screening with improved strategy to evaluate the efficacy of various potential plants against problematic multi drug resistant bacteria is in the stage of infancy.

It is expected that plant extract showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However very little information is available on such activity of plant extract (Lee *et al.,* 1998). The selection of Herbal ingredients were based on their traditional uses in India and reported antimicrobial activity of many medicinal plants (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et. al.,* 1999).

In the recent years, based on leads from Ayurvedic system and other traditional medicine system, several antiviral agents have been isolated from plants. These agents include a variety of polyphenols, flavonoids, saponins, glucosides, and alkaloids. Some traditional Indian spices and herbs are taken as ingredeints in immunity booster formulation IMBF-01-20 with an aim to prepare the body to fight infection.

The search for natural antioxidants has generated interest among scientific community to reinvestigate

Indian Herbs as safe and promising antioxidant agent (Wannes et al., 2010; Tongpoothorn et al., 2012; Kapoor et al., 2014).

The current research in the area to develop plant-derived natural products as potent and safer leads to act as Generation of herbal medicine as multiple-component agent expected to modulate the complex immune process in such a way so as to prevent the infection rather than treatment and cure of the disease. With all these aspects keeping in mind, the present work focuses on an immunobooster tea formulation with its *in vitro* antimicrobial and antioxidant activity.

Material and methods

Plants material

The authentic plant/Herbs were obtained from the Himalaya Drug Company, Dehradun. The identification of the samples was further confirmed by the plant taxonomist, Dr. Maya Ram Uniyal Former Jaribooti Expert Govt.of Uttarakhand, Dehradun. The voucher specimen has been deposited in the Herbal Museum The Himalaya Drug Company Dehradun Uttarakhand.

Bacterial strains used in the screening programme

The Standard strains were obtained from HiMEDIA**,** Mumbai include the ATCC strains of *Staphylococcus aureus* (MRSA), *and* strains of *E. coli.*

Culture Media and Inoculum preparation

Nutrient broth/ Agar and MullerHinton broth/ agar (Hi-Media Pvt. Ltd., Mumbai, India) were used to grow the test bacteria at appropriate temperature 30-37 ^{0}C for 18hrs and then appropriately diluted in sterile 0.8% saline solution to obtain a cell suspension of 10^{5} 10^{6}CFU/ml.

Preparation of plant extracts and its fractionation

The extract of IMBF-01-20 was prepared as described earlier (Ahmad and Beg 2001) with a little modification. 800 gram of dry, powder was soaked in 1.5 liter each of Methanol, Hexane and 70% ethanol, for 36 days and stirred after every 10 hr using a sterilised glass rod. At the end of extraction, it was passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates were concentrated under vacuum on rotary evaporator at 40 °C and then stored at 4 °C for further use. The crude extract was prepared by dissolving known amount of the dry extract in DMSO(Dimethylsulpoxide), to have a stock solution of 100 mg/ml concentration.

Antimicrobial assay

The agar well diffusion method (Perez et al. 1990) as adopted earlier (Ahmad and Beg, 2001) was used. 0.1 ml of diluted inoculum (10^{5} CFU/ml) of test organism was spread on Muller-Hinton agar plates. Wells of 8 mm diameter were punched into the agar medium and filled with 100µl of plant extract of 10mg/ml concentration and solvent blank (DMSO) separately. The plates were incubated at 37 °C, over night. The antibiotic (chloramphenicol) at 100µg/ml conc. was used in the test system as positive control. Zone of inhibition of bacterial growth around each well was measured in mm.

DPPH radical scavenging assay

Free radical scavenging activity of Immunobooster tea formulation against stable 1, 1,diphenyl-2-picrylhydrazyl (DPPH) was determined spectrophotometrically by slightly modified method of Gyamfi et al. (1999) as described below. When DPPH reacts with an antioxidant, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm on a UV/visible light spectrophotometer (Spectronic 20 D+, Thermo Scientific, USA). Fifty µl of Immunobooster tea extract in DMSO, yielding different concentrations was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mMTris-HCl buffer (pH 7.4). DMSO (50 µl) was used as a vehicle control in the experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured spectrophotometrically. Ascorbic acid and butylated hydroxytoluene were used as positive controls. Inhibition percent was calculated from the following equation:

% Inhibition = [Absorbance of control Absorbance of test sample / Absorbance of control] × 100

Results and Discussion

In the present study, Herbs were selected on the basis of their traditional uses in treatment of different diseases in India and worldwide. Only alcoholic extracts of Immunobooster tea formulation have been used as the alcohol was found suitable solvent for the extraction of antimicrobially active constituents from plants (Eloff,1998;Ahmad et al.,1998).

Antibacterial activity of Immunobooster tea against Gram positive bacteria (*S. aureus ATCC- 6538*) and Gram- negative bacteria (*E. Coli*, ATCC-8739) is presented in Table-1, Plate No-1-5. Activity of Methanolic extracts against Gram positive bacteria showed strong zone of inhibition of 32 mm followed by 20 mm against Gram negative bacteria(Plate-1&4). On the other hand Hexane extract showed 19mm and14mm against gram positive and gram negative respectively Followed by Ethanol extract. Most potential extract was methanol extract followed by Hexane and Ethanol as depicted in plate-1 to 5.

Antioxidant activity

The immunobooster tea formulation IMBF-01-20 under study was subjected to antioxidant scrutiny by standard methods namely DPPH free radical scavenging activity .The sample was ten times diluted in dimethyl sulpho-oxide and were tested at concentration ranging from 100-500 µg/ml. IMBF-01-20 demonstrated strong DPPH scavenging activity (>93.54% decolorization) at 500 µg/ml concentrationas shown in Table-2. The values were comparable to commercial standards ascorbic acid (94.9%) and BHT (92.8%).

The antioxidant potential of essential oils highlights their therapeutic potential both in traditional and modern phytomedicine. Since essential oils in herbs/culinary herbs and spices consist of terpenes and other different kind of phenolic compound, it would seem reasonable that their mode of action might be related to those of other phenolic compounds (Tassou et al., 2000) and contribute to their antioxidant activity.

Our findings are in agreements with reports of various other workers who have investigated for the similar antioxidant potential of essential oils and their active compounds from India as well as other parts of the world (Dorman et al., 2000; Manuel et al., 2010; Zahin et al.,2010; Vaibhavi et al., 2010). However, the activities slightly differ due to difference of minor phytoconstituents and the variability in the quantity of major active constituents which may arise due to different agroclimatic condition, plant varieties and extraction processes.

Various herbal medicines have been found to modulate various components of innate and acquired immune system. In fact, based on proper understanding of various immunomodulatory activities of herbal plants, plants derived the secondary metabolites in natural products can be the lead molecules for the future development of immunomodulators for therapeutic use. Various immunomodulators have been suggested in various allergic diseases including asthma, allergic rhinitis, and eosinophilic esophagitis on the basis of experiments performed on various animal models.

Conclusion

On the basis of preliminary investigation of the present study, it may be concluded that the tested immunobooster tea formulation have the potential for application in real food system and healthcare.

This preliminary investigation indicated that potential plants/herbs extracts showing broad spectrum antimicrobial activity could be further tested to determine the efficacy in-vivo against MDR bacteria. Active fractions of various plants may also be exploited in preparation of herbal formulation of improved efficacy and quality.

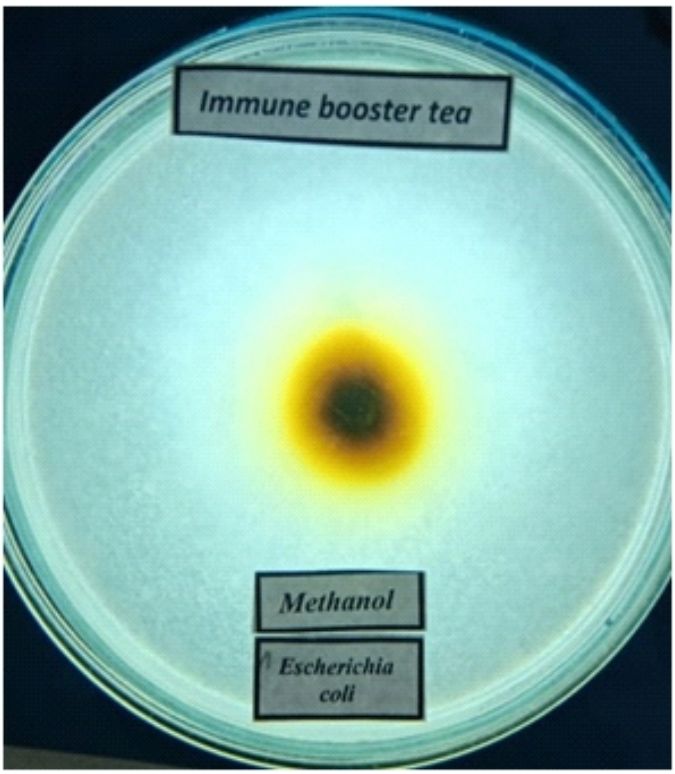
Many of the chemicals in the form of alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products are responsible to cause alterations in the immunomodulatory properties.

Table-1: Antimicrobial activity of Immunobooster tea formulation

S.No.	Extract	Diameter of zones of inhibition(mm)		
		Staph.aureus	*E.coli*	Ciprofloxacin (as positive control)
1.0.	Methanol	32	20	34 for *Staph aureus*and 33 for *E.coli*
2.0.	Hexane	19	14	
3.0.	Ethanol	17	15	

Table-2 Antioxidant activity of Immunobooster tea Formulation

S.No.	Test sample	Antioxidant activity in %(DPPH method)
1.0.	Immunobooster tea Sample	93.54%
2.0.	Ascorbic Acid	94.9%
3.0.	BHT	92.8%.



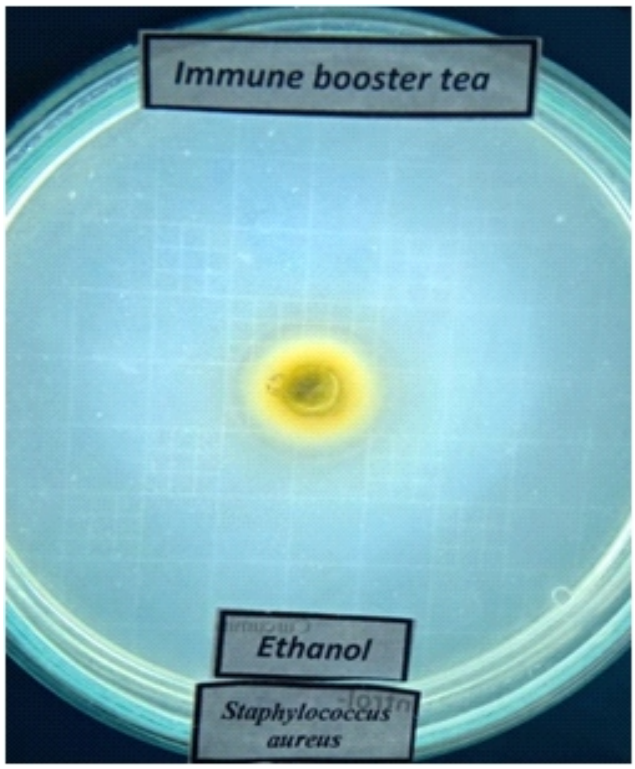
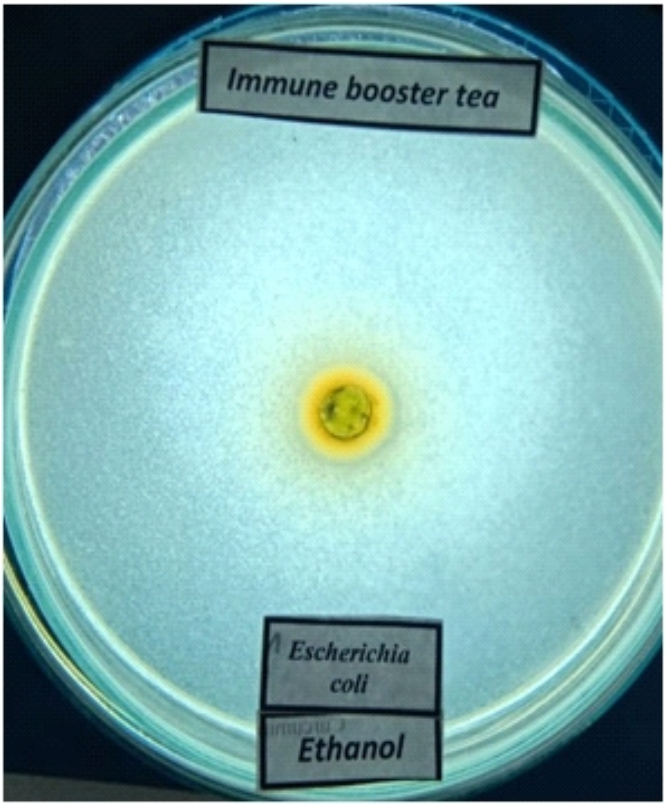


PLATE-1 Antimicrobial activity of methanol extract against EC
PLATE-2 Antimicrobial activity of ethanol extract against SA



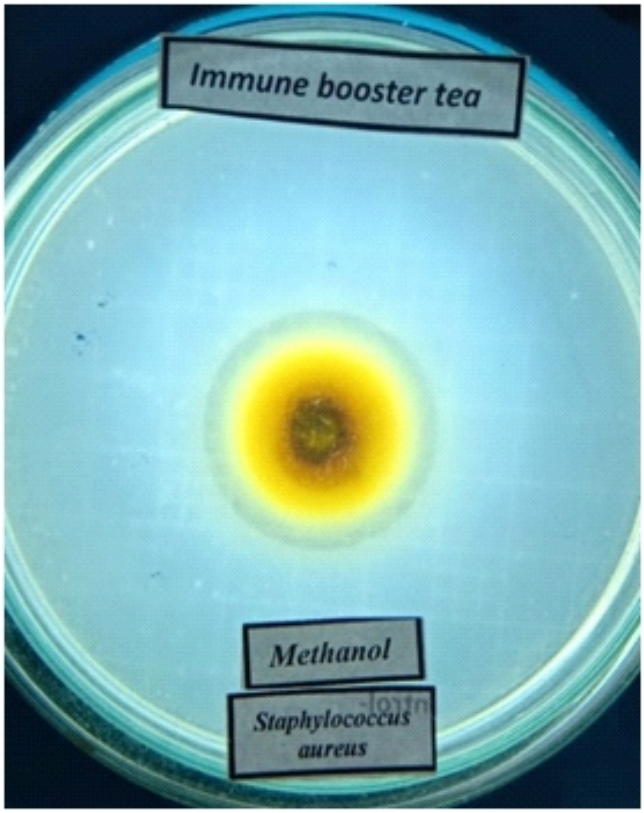


PLATE-3 Antimicrobial activity of ethanol extract against EC
PLATE-4 Antimicrobial activity of methanol extract against SA

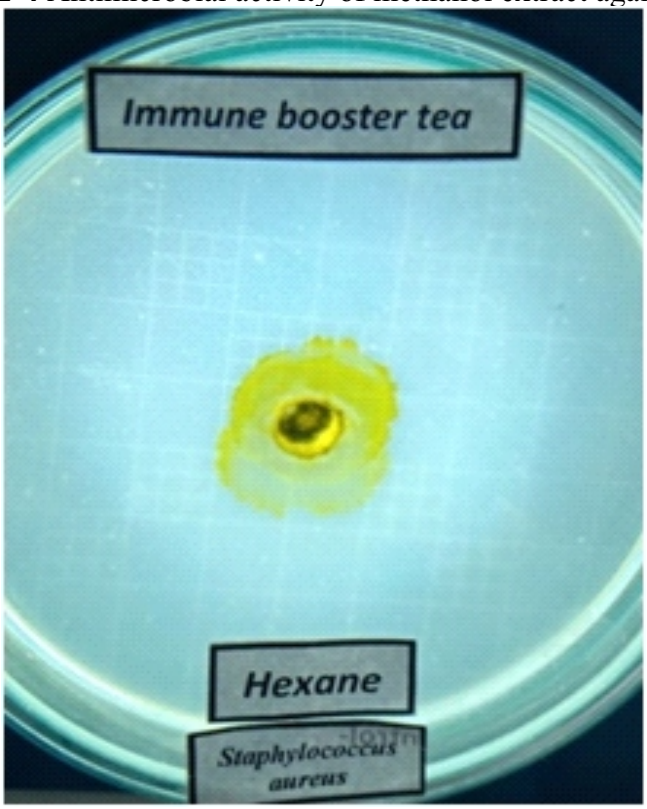


PLATE-5 Antimicrobial activity of Hexane extract against SA
Note: EC; *E. Coli* & SA; *Staph aureus*

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