Comparative Pharmacological Assessment of Polyherbal Formulation Immunoboost Tablet and Immunoboost Herbal Extract Capsule

[1]Renu Kaushik, [2]Amrita Singh, [3]Mohd Zafar, [4]Bhanu P. S. Sagar

IEC Department of Pharmacy
IEC Group of Institutions
Greater Noida, Uttar Pradesh, India

Abstract- **In current scenario, more than 500 Immunomodulatory phytoformulations are globally commercial available either online or offline for prophylactic use (food supplements) therapeutic use (immunomodulatory) to strengthen the body immunity. At yet, Immuno Boost Tablet (IBT, NatureFlip) and Immuno Boost Herbal Extract Capsule (IBHEC, Medinutrica) are not scientifically pharmacologically assessed for their immunomodulatory and other pharmacological investigations. So, present investigation was an attempt to investigate pharmacological potential of IBT andIBHEC. IBT showed 94 SPMs in dried aqueous extract / powder of 12 plants (Haritaki, Amla, Baheda, Haldi, Kali Mirch, Pippali, Guduchi, Yastimadhu / Mulethi, Tulsi, Chitrak, Sounth and Manjishta) whereas IBHEC indicated 44 SPMs in dried aqueous extract of 05 medicinal plants (Amla, Tulsi, Giloy, Haldi and Mulethi). On comparison, IBT was found to be more powerful immunomodulatory then IBHEC. Besides, both IBT and IBHEC were found to be non-toxic / non-lethal (no mortality found even at dose of 2000 mg/kg) with wide safety margin (free from side effects / lethal toxicity). Further, both formulations indicated presence of PPMs and SPMs of allcategories and due to high phenolic and flavonoid content in IBT it showed high TPC and TFC content and better antioxidant properties then IBHEC. Both IBT and IBHEC showed good adaptogenic properties and also produced significant anti-inflammatory effects (decrease in edema) when compared with Indomethacin (standard drug).**

Keywords: **Antioxidant, anti-inflammatory, Immunomodulators, Immunosuppressants, Immunoadjuvants, total phenolic content, total flavonoid content.**

Introduction

Immune system and Immunomodulators

Agarwal and Singh (1999), immune system is multilayered with several levels defenses and immunomodulators are substances (natural or artificial) that help to regulate (stimulate, suppress, or modulate both innate adaptive arms of the immune response) the immune system.

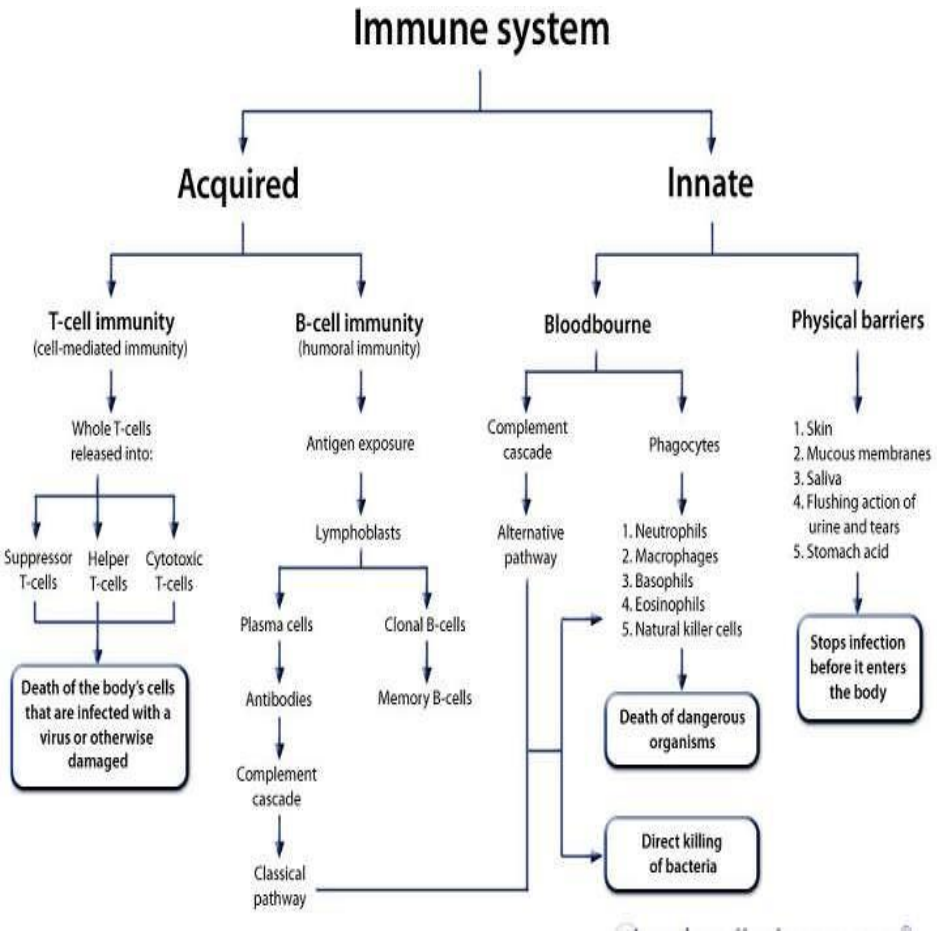


Figure 1: Immune System.

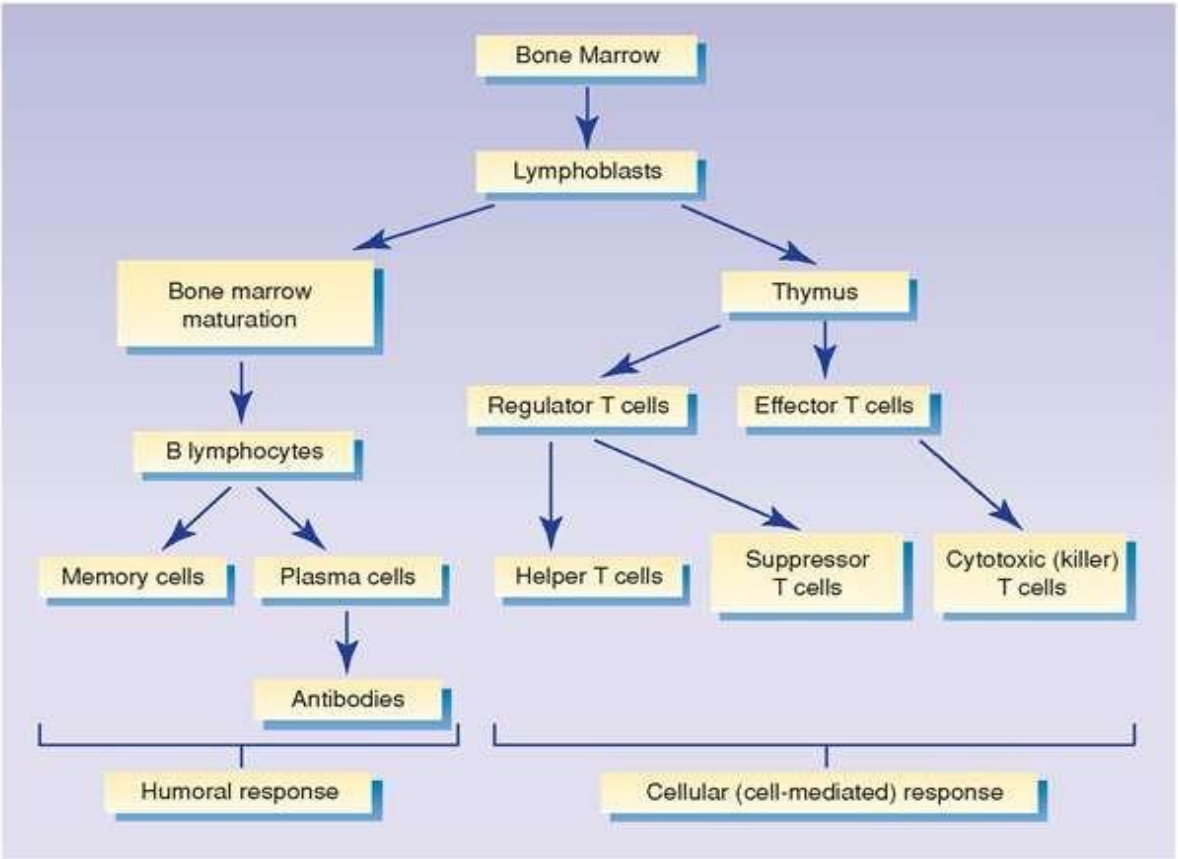


Figure 2 : Development of the cells of the immune system.

Immunomodulators Types (03 Types)

Immunoenhancers / Immunostimulants

Juyal & Singla (2007), enhance or stimulate the immune system (immunopotentiators).

Immunosuppressants

El-Sheikh (2008), agents which suppress the immune system (autoimmune diseases).

Immunoadjuvants

Alfons and Patrick (2001), immunoadjuvants are true modulators of the immune response and enhance efficacy of vaccines.

Herbal Medicines as Immunomodulatory drug

Ahmed *et al*., 2021, now days, there is a strong demands of immunomodulatorydrugs to combat chronic diseases like neurodegeneration, cancers, HIV and diabetes. These are various herbal drugs, SPMs and herbal formulations with immunomodulatoryactivity (Shi *et al*., 2021; Zarrin *et al*., 2021).

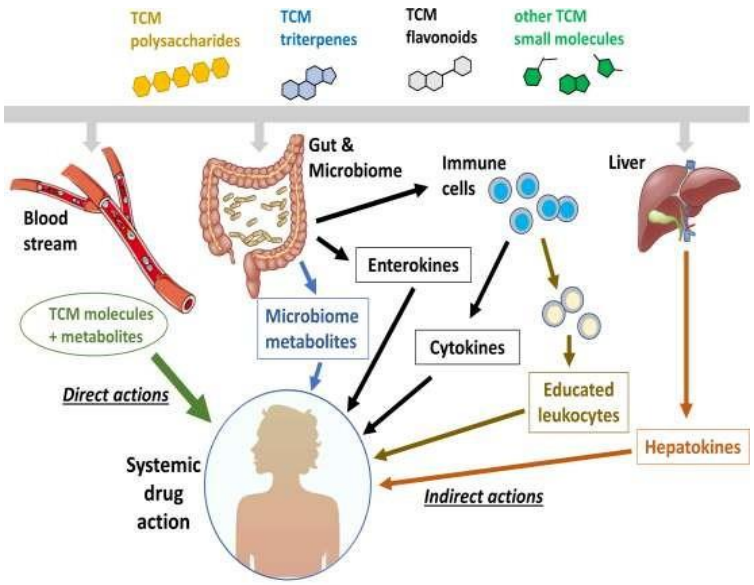


Figure 3: Systemic action of plant derived immunomodulatoty drugs.

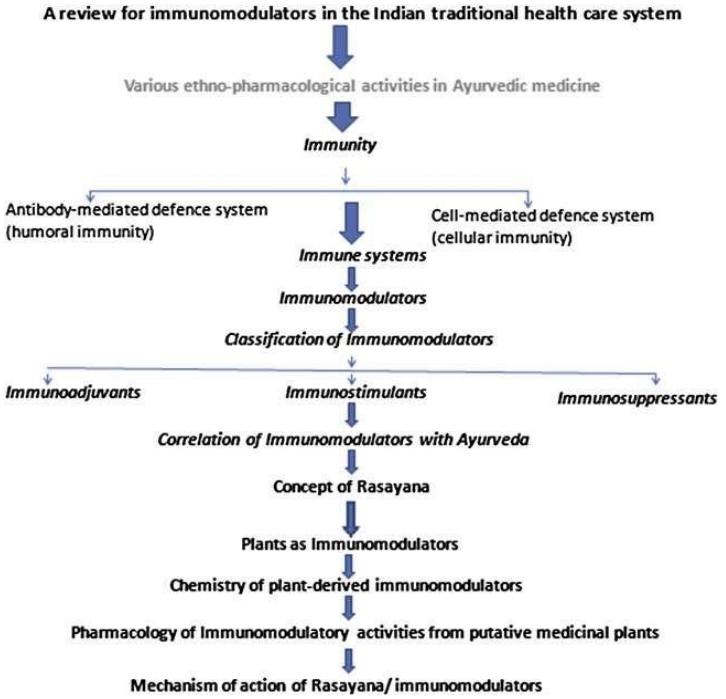


Figure 4 : Plant SPMs derived immunomodulation.

There are various categories of medicinal plants constituents / phytotherapeutic agents possess immunomodulatory activity and used for pharmacological purposes.

Glycosides

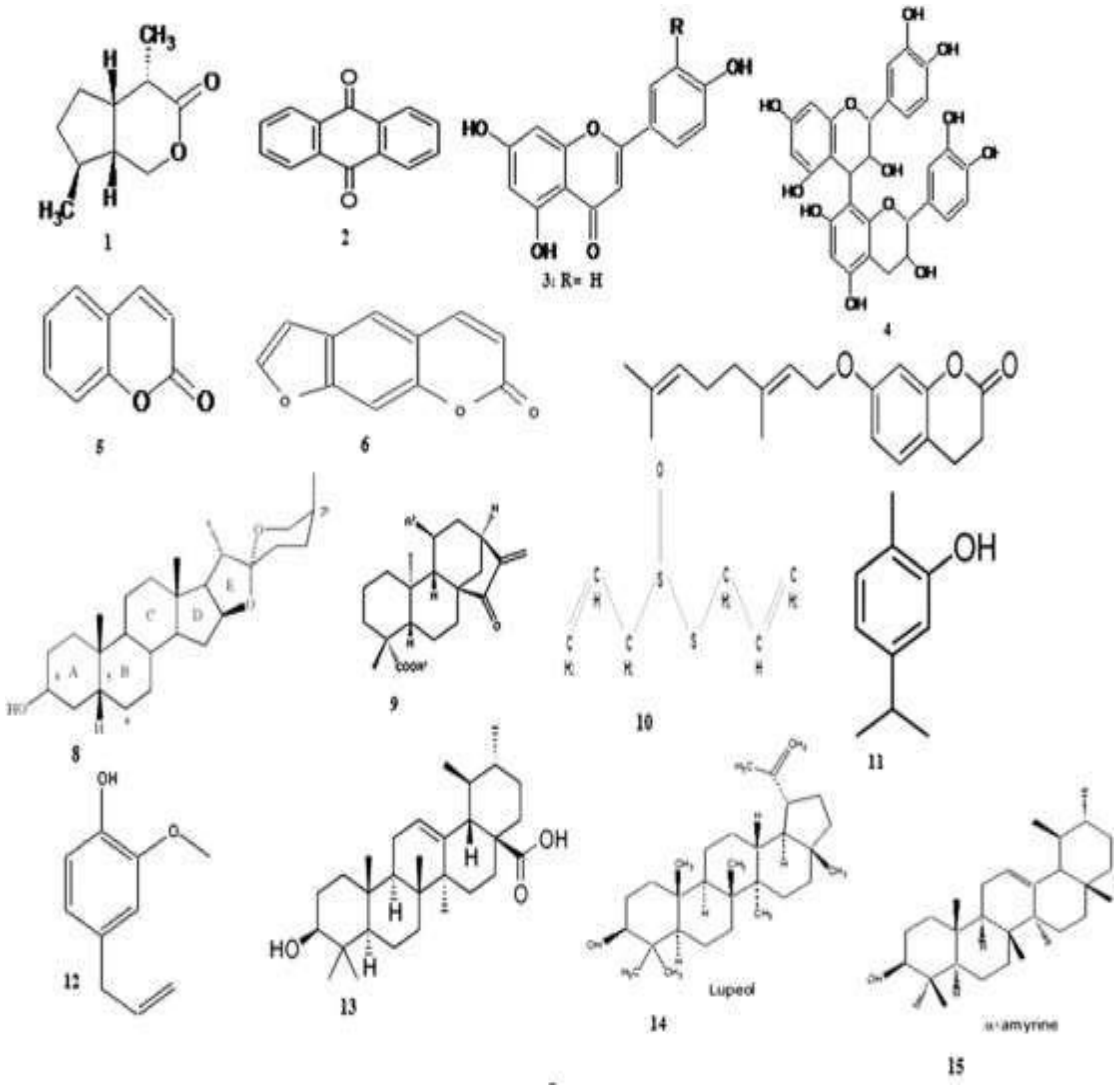


Figure 5 : Bioactive molecules showing immunomodulatory activity.

Alkaloids

Alkaloids (heterocyclic e.g. *Murraya koenigii*, *Achillea millefolium*, *Actinidia macrosperma*, and *Cissampelos pareira*). Compounds like Piperidine, sinomenine, berbamine, tetrandrine, dauricine, hemandezine are few alkaloidal compounds which possess immunomodulatory properties.

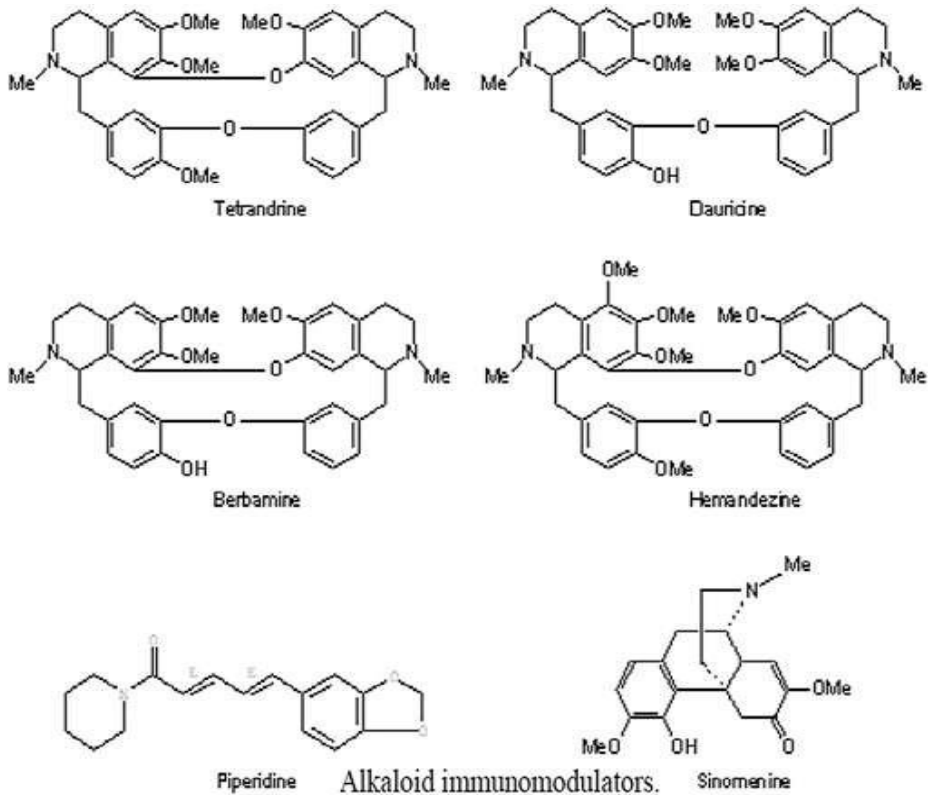


Figure 6: Alkaloidal SPMs as immunomodulatory agents.

Flavonoids : Flavonoids (C6-C3-C6; fifteen carbon skeleton; two phenyl rings connected bya three-carbon bridge) possess exert immunomodulatory activities (e.g. isoflavonoids, apigenin, flavones, anthocyanidins, oligomeric proanthocyanidins).

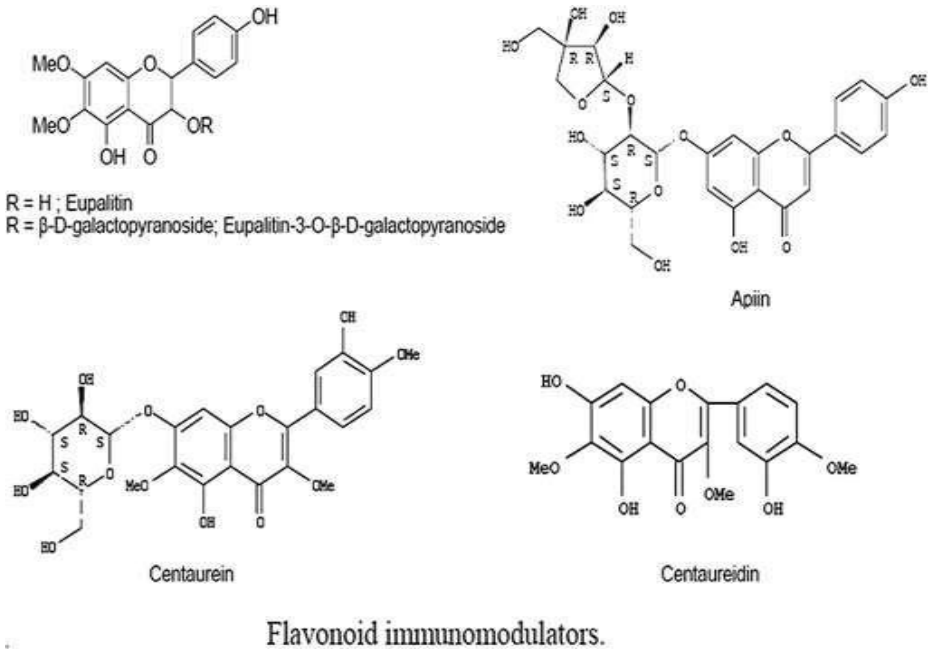
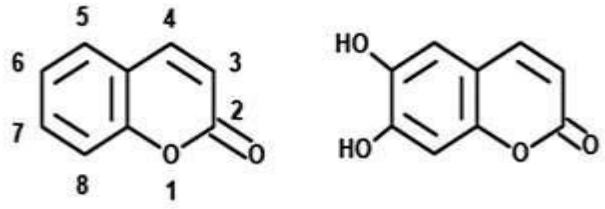


Figure 7: Flavonoids as immunomodulatory agent.

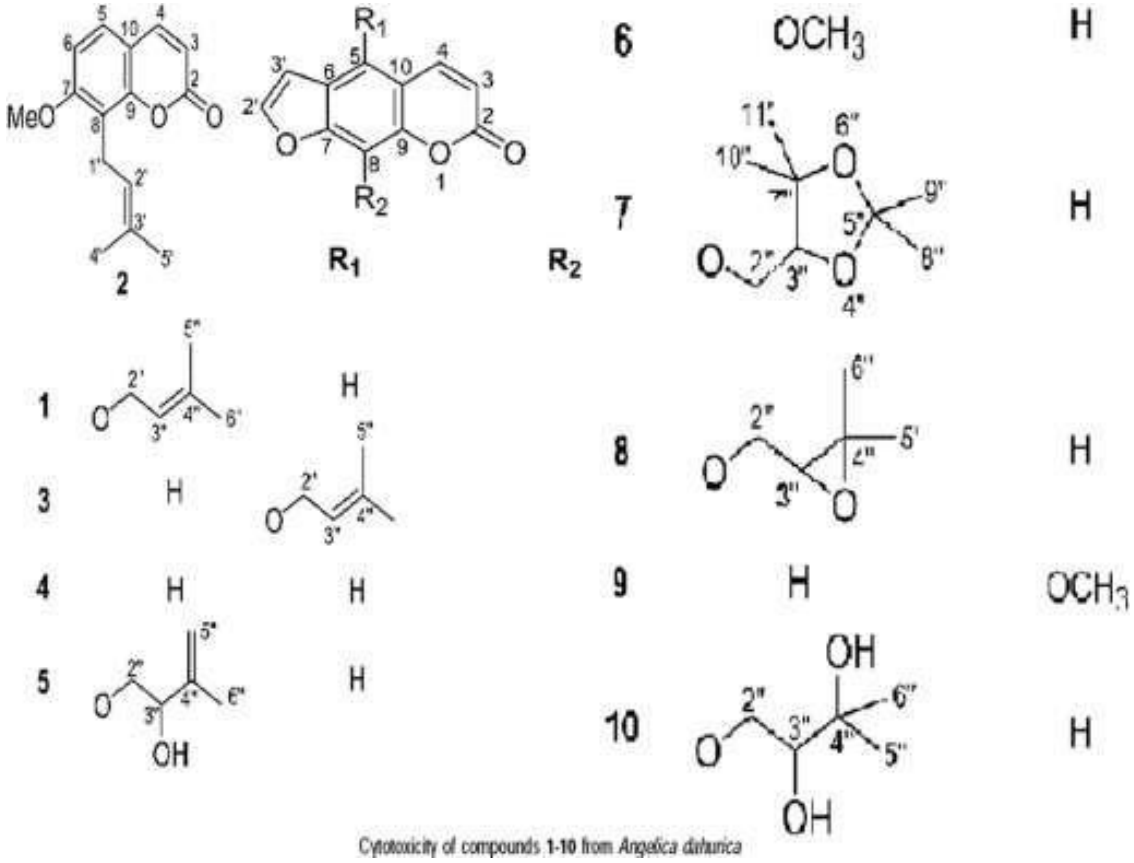
Coumarins

Coumarins glycosides possess immunomodulatory activities (e.g. *Heracleum persicum*, *Achillea millefolium*, *Citrus limonia*, *Artemisia capillaries*, *Citrus natsudaidai*, *Euphorbia lathyris*). They possess antitumor activity etc. (Figure 8-9).



Chemical structures of coumarin and esculetin (6,7- dihydroxycoumarin).

Figure 8: Esculetin (6, 7- dihydroxycoumarin).



Cytotoxicity of compounds 1-10 from *Angelica dahurica*

Figure 9: Compound with immunomodulatory potential.

Cucurbitacin

Lagenaria siceraria leaves, fruit, possess immunomodulatory properties.

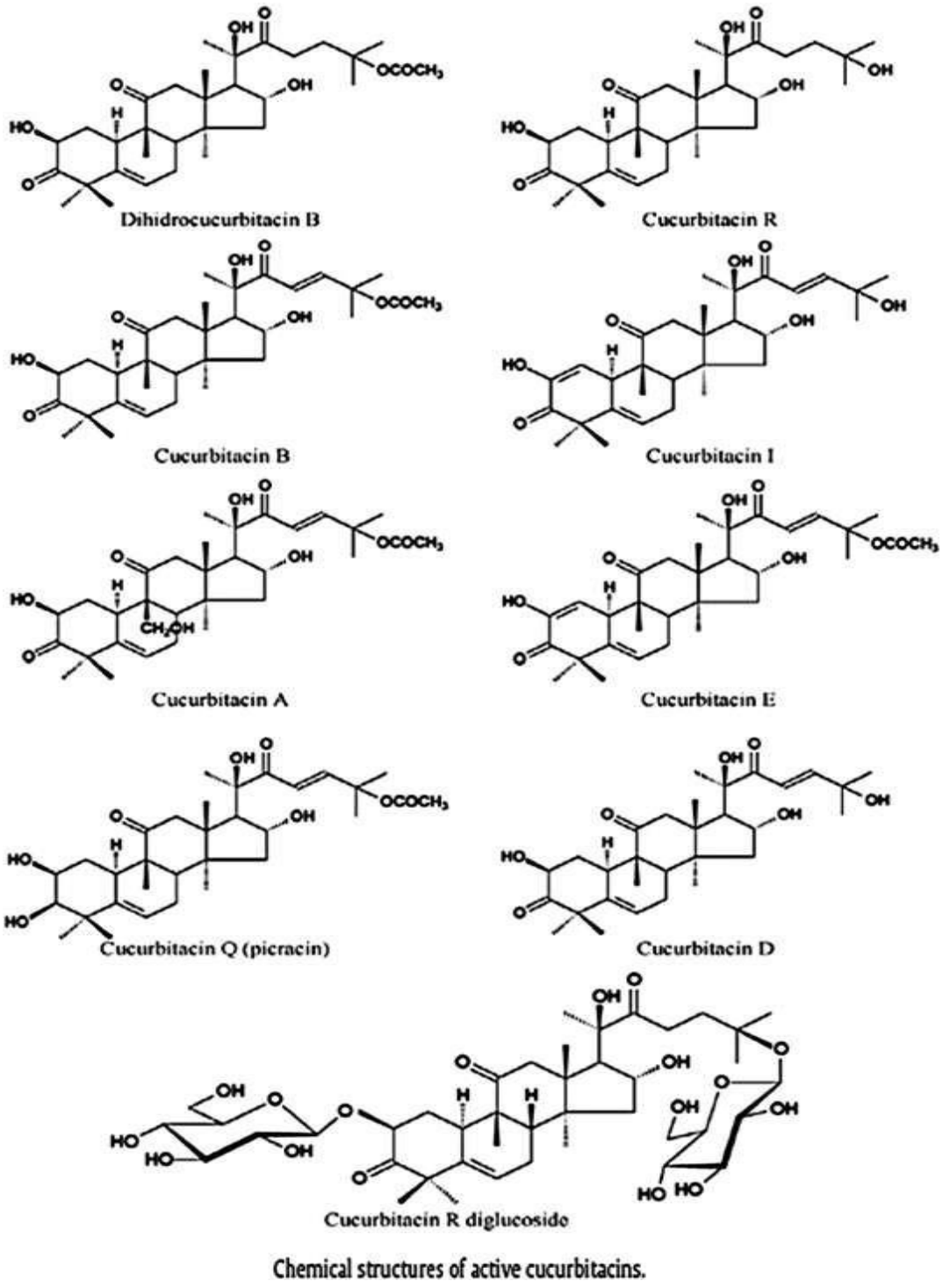


Figure 10: Cucurbitacins as immunomodulatory agents.

Volatile oils and terpenoids

Terpene (C_5H_8; hydrocarbons and oxygenated derivatives) of volatile oils of plant / animal exhibit immunomodulatory activity eg. Carvacrol; eugenol; diterpene; triterpenes; lupeol and amyrine.

Sapogenins

Sapogenins (triterpenoid saponins and diterpenes) exert immunomodulatory activities (eg. *Chlorophytum borivilianum*, *Gymnema sylvestre*, *Boswellia* spp. and*Randia dumetorum*).

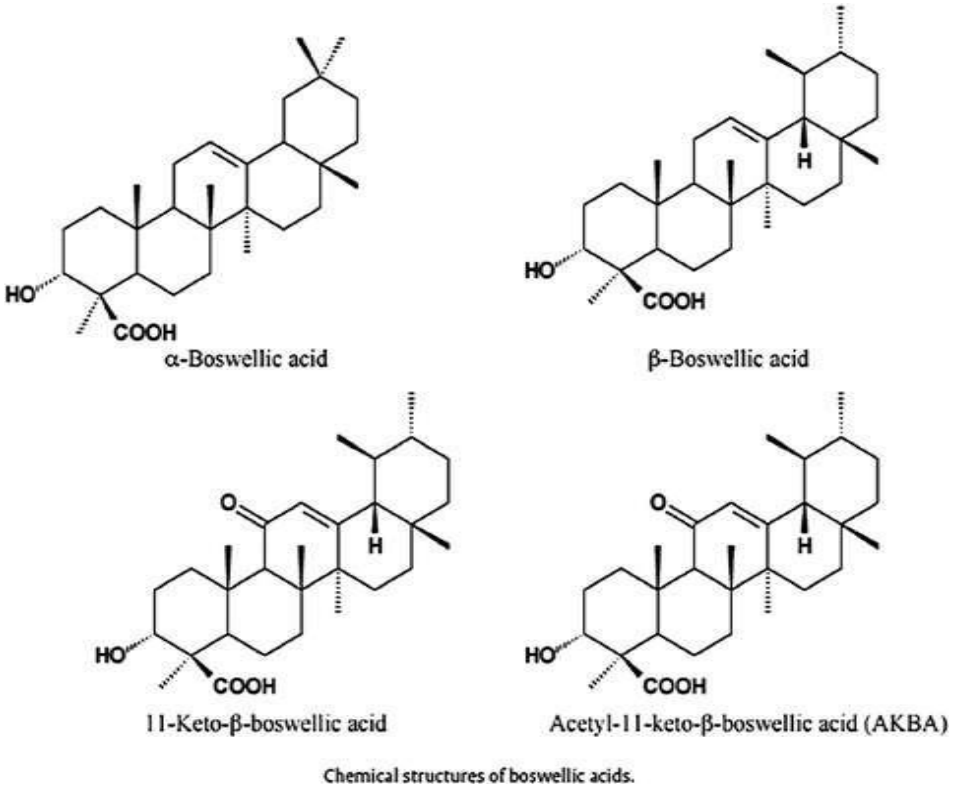


Figure 11: Boswellic acids from *Boswellia* spp. as immunomodulatory agents.

Polyherbal Formulation Immunoboost Tablet (IBT, NatureFlip)

Immuno boost is a blend of herbs which acts as an Immuno-Modulator to fight againstdisease causing bacteria and other such micro-organisms. It's anti-oxidant, anti-bacterial, anti-microbial & anti-infective properties acts asupport for your immune system. Its improves heart health Purifies blood.

Figure 12 : Immunoboost Tablet (IBT, NatureFlip)**.**

Ingredients of Immunoboost Tablet (IBT, NatureFlip) :

i. Haldi (Turmeric *Curcuma longa* L.; Zingiberaceae) (Khalander *et al*., 2018)
ii. Guduchi (*Tinospora cordifolia* Linn.; Giloy, Menispermaeceae)
iii. Tulsi (Holy basil; *Ocimum sanctum*; Family-Lamiaceae)
iv. Yastimadhu (*Glycyrrhiza glabra*, Fabaceae, Mulethi / liquorice)

v. Haritaki (Myrobalan *; Terminalia chebula*; Family: Combretaceae)
vi. Baheda (Myrobalan, *Terminalia bellirica,* Family : Combretaceae)
vii. Amla (*Emblica officinalis*, Family: Euphorbiaceae, Indian Gooseberry)
viii. Kali Mirch (King of Spices, *Piper nigrum*, Family:Piperaceae) (Jaramillo *et al*., 2001)
ix. Manjishta (Indian Madder, *Rubia cordifolia,* Family: Rubiaceae)

Polyherbal Formulation Immunoboost Herbal Extract Capsule (IBHEC)

Medinutrica Immuno Boost Capsule is a powerful herbal immunity booster with potent antioxidants & essential minerals that are required to strengthen theImmune system naturally. It contains extracts of special herbs like Tulsi, Amla,Mulethi, Giloy & Harad in proper doses that helps to improve body's resistance power and strength.

Figure 13 : Immuno Boost Herbal Extract Capsule (IBHEC, Medinutrica).

Acharya and Shrivastava (2008), immune system protect the body from invading infectious pathogens (potentially harmful microorganisms) and eliminate disease. Immunomodulators (biological or synthetic) regulate the immune system. (Agarwal and Singh, 1999). Thatte and Dahanukar (1997), antibody-mediated defense system (humoral immunity) and by the cell-mediated defense system (cellular immunity).

Premanathan *et al*., 2000, traditional and herbal medicines continue to significant and vital roles in health services throughout the world. Herbal SPMs (proteins, flavonoids, alkaloids, terpenes, resins, coumarins, sterols, steroids, glycosides, polyphenols) immunostimulants, immunoadjuvants, and immunosuppressants are useful (as strong antioxidant) in rheumatoid arthritis, ageing, diabetes, atherosclerosis, cancer, Parkinson's disease and autoimmune disease. (Alamgir and Uddin, 2010)

Wagner (1984), MoA of immunomodulation by phagocytosis stimulation; macrophages activation; lymphoid cells stimulation; immunostimulatory effect on peritoneal macrophages; antigen-specific immunoglobulin production increase; cellular immune function enhancement and nonspecific cellular immune system effect. (Xiu *et al*., 2007)

Immuno Boost Tablet (Ingredients like Haldi, Guduchi, Tulsi, Yastimadhu, Haritaki, Baheda, Amla, Kali Mirch and Manjishta; Nature Flip) is a polyherbal formulation acts as Immunomodulator and useful as anti-oxidant, anti-bacterial, anti-microbial & anti-infective agent. Further, Immuno Boost Capsule (Tulsi, Amla, Mulethi, Giloy & Harad; Medinutrica) possess antioxidants & essential minerals which strengthen immune system. Immunoboost Tablet (IBT) and Immunoboost Herbal Extract Capsule (IBHEC) were pharmacologically investigated with following objectives:

- *In-vitro* antioxidant activity of IBT (Nature Flip) and IBHEC (Medinutrica).
- Adaptogenic property of IBT and IBHEC.
- *In vivo* immunomodulatory activity of IBT and IBHEC.
- Safety and toxicity evaluation of polyherbal formulations IBT and IBHEC.
- Effects of IBT and IBHEC on blood parameters, LFT, KFT, triglycerides,cholesterol for acute and sub-acute toxicity studies.
- Anti-inflammatory activity of IBT and IBHEC.

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Materials and Methods

- Modern scientific techniques of phytochemical screening;
- *In-vitro* antioxidant property of IBT and IBHEC by NO free radicalscavenging.
- Acute toxicity evaluation by estimation of biochemical and behavioural analysis.
- *In-vivo* immunomodulatory activity of IBT and IBHEC
- *In-vivo* anti-inflammatory activity by Carrageenan Induced Rat Paw Edema in Rats.

Pharmacognostical and Pharmacological Assessment of IBT and IBHEC
Collection of Materials, Chemicals and Drugs

Immuno Boost Tablet (IBT, Nature Flip) and Immuno Boost Capsule (IBHEC, Medinutrica) were procured from concerned companies and reliable commercial source and analytical grade chemicals and reagents were used in the different studies.

Composition of Immunoboost Tablet (Nature Flip)

Table 1: Composition of Immunoboost Tablet (NatureFlip; 500 mg each tablet)

Constituents	Part used	Quantity (mg)
DAE of Haldi (*Curcuma longa*)	Rhizomes	100
DAE of Guduchi (*Tinospora cordifolia*)	Stem	180
DAE of Tulsi (*Ocimum sanctum*)	Leaf	50
DAE of Mulethi / Yashtimadhu (*Glycyrrhiza glabra*)	Stem	20
DAE of Haritaki (*Terminalia chebula*; Myrobalan)	Fruit	20
DAE of Baheda (*Terminalia bellirica*; Combretaceae)	Fruit	20
DAE of Amla (*Emblica officinalis*; Indian Gooseberry)	Fruit	20
DAE of Manjishta (*Rubia cordifolia*; Indian Madder)	Root	20
Powder of Chitrak (*Plumbago zeylanica*)	Root	20
Powder of Pippali (*Piper longum*)	Fruit	20
Powder of Kali Mirch (*Piper nigrum*; Black Pepper)	Fruit	20
Powder of Sonth (*Zingiber officinale*; Adrak)	Rhizome	10
Excipients : Quantity Sufficient		q.s.
Total		500
Preservatives : Sodium methyl paraben; Sodium Propyl Paraben;		

Table 2 : SPMs of Immunoboost Tablet (IBT, NatureFlip).

S. No.	**Constituent**	**Source**	Uses
1.	Curcumin	*Curcuma longa* (Haldi) Zingiberaceae	Skin disorders, Respiratory tract diseases, antimicrobial, arthritis, anti-inflammatory, liver toxicity, Antioxidant, Alzheimer's disease, anti- aging, Immunobooster, heart diseases, imrove digestion, in glaucoma.
2.	Curcuminoids		
3.	Vanillic acid		
4.	p-Coumaric		
5.	Zingiberene		
6.	Eucalyptol		
7.	Borneol		
8.	Sabinene		
9.	P-cymene		
10.	Glycyrrhizin	*Glycyrrhiza glabra* (Mulethi) (Fabaceae) liquorice"	Antiviral, antimicrobial, lung diseases, gastric ulcer, low blood pressure,allergies, liver toxicity,
11.	licoricidin		
12.	glycyrrhizic acid		
13.	18β- Glycyrrhetinic acid		
14.	Licochalcone		
15.	Glabridin		
16.	Isoliquiritigenin		
17.	Eugenol	*Ocimum basilicum* (Tulsi) (Family-Lamiaceae)	Immunno-stimulatory; Enhance secretion of phlegm (relieve cough); Anti-inflammatory; Anti-microbial; Adaptogenic; Improve Digestion; Reduce Stress; Antioxidant; Anti-microbial; Cure Respiratory diseases;
18.	methyl cinnamate		
19.	Linalool		
20.	β-elemene		
21.	Camphor		
22.	Linalyl acetate		
23.	ß- Ocimene		
24.	Eucalyptol		
25.	Neral		
26	Geranyl acetate		
27	Cyclohexanemethanol		
28.	Trans-caryophyllene		
28	Cyclopentylacetone		

29.	Piperine	*Piper nigrum* (Piperaceae) (Marich) King of Spices/ Black Pepper	Cytotoxic, anti-inflammatory, anti-mycobacterial, insecticidal, anti-protozoal, analgesic, anxiolytic, antidepressant activities, Radical scavenging, antioxidant, allelopathy, anti-convulsant, anti-tubercular, antioxidant, antibacterial, antipyretic, exterofective;
30.	Piperettine		
31.	Piperolides		
32.	Propenylphenols		
33.	Coumaperine		
34.	Pipernigramide A		
35.	Piperanine		
36.	Achilleamide		
37.	Butylated hydroxytoluene (BHT)		
38.	Alpha-tocopherol		
39.	Butylated hydroxyanisole		
40.	Piperic acid		
41.	Caryophyllene		
42.	Camphene		
43.	Limonene		
44.	Berberine	*Tinospora cordifolia* Linn.(Giloy)	Palmatine; insulin mimicking effect; Jatrorrhizine; mimicking effect; Magnoflorine;Insulin release
45.	Palmatine		
46.	Jatrorrhizine		
47.	Magnoflorine		
48.	Jateorine		
49.	Phenolics: chebulinic acid, ellagicacid,	Haritaki *(Terminaliachebula)* (Myrobalan) (Combretaceae) "King of all medicine"	Antioxidant, Anti-cancinogenic activity. Anti-bacterial, anti-mutagenic, Cardioprotective Chemoprotective, anti-inflammatory, Hepatoprotective, Anti-arthritic
50.	Polyphenols : corilagin, punicalagin		
51.	Flavonols		
52.	Anthraquinones		
53.	Glycosides: chebulosides		
54.	Triperpenoids		
55.	Tannins: Gallic acid,		
56.	Caffeic acid		

57.	Vanillic acid		
58.	Palmitic acid; linoleic acid		
59.	Tannins	Baheda (*Terminaliabellirica*) Myrobalan (Combretaceae)	Improve appetite, obesity, wound healing, antioxidant, anti-diabetic, anti-cancer, Hepatitis, dyspepsia, hair tonic, menstrual disorders
60.	Pseudo-tannins		
61.	Gallic acid, chebulinic acid, Chebulagic acid		
62.	Corilagin		
63.	Triterpenes, Triperpenoids		
64.	Gallic acid	(*Phyllanthus emblica / Emblica officinalis*) Amla / Indian Gooseberry (Euphorbiaceae; Phyllanthaceae)	Antioxidant, Analgesic, anti-inflammatory, hypolipidaemic, chemoprotective, Antidiabetic, Hepatoprotective,t anti- HIV-1, Anticancer, Immunomodu-latory, Cardioprotective.
65.	Tannin (ellagitannins)		
66.	Phyllemblin		
67.	Flavonoids		
68.	Kaempferol		
69.	Vitamin C		
70.	Polyphenols		
71.	Amino acids (Glutamic acid, aspartic acid, alanine, lysine)		
72.	Diterpenes, triterpene		
73.	Phyllantine		
74.	Pseudopurin	Indian Madder *Rubia cordifolia* (Manjishta) (family: Rubiaceae)	Immunomodulatory, anti-inflammatory, skin complexion, acne infections, Urolithiasis, antibacterial antioxidant.
75.	Purpurin		
76.	Manjistin		
77.	Rubiadin		
78.	Xanthopurin		
79.	Triterpenoids	Chitrak (*Plumbagozeylanica*)	Anti-cancer, hepatoprtective, antioxidant, Immunostimulatory,
80.	Flavonoids		
81	Phenolic		
82	Tannins,		
83	Saponins		
84.	Alkaloids (Piperine)	Pippali (*Piper longum*)	Respiratory disorders, Antioxidant Anti-ulcer, Anti-amebic,
85.	Volatile oil		
86.	Isobutylamides		

87.	Lignans		Immunostimulatory, Hepatoprotective, Anti-inflammatory
88.	Esters		
89.	Sesquiterpenes	Sonth / Ginger (*Zingiber officinale*)	Anticancer, Antidiabetic, nephroprotective, hepato-protective, larvicidal, analgesic, Antioxidant, Anti-inflammatory, immunomodulatory, Radioprotective.
90.	Zingerone		
91.	Gingerols		
92.	Phytosterols		
93.	Zingiberene		
94.	Shogaols		

Immunoboost Tablet (500 mg Tablet; NatureFlip) contains dried aqueous extract (DAE) of Haldi, Guduchi, Tulsi, Yastimadhu, Haritaki, Baheda, Amla, Chitrak, Sounth,Pippali, Kali Mirch and Manjishta with more than 94 phytotherapeutics / SPMs derived from 12 medicinal plants. (Table 3)

Composition of Immunoboost Herbal Extract Capsule (Medinutrica)

Table 3: Composition of Immuno Boost Herbal Extract capsule (500 mg)

Constituents	Part used	Quantity (mg)
DAE Guduchi (*Tinospora cordifolia*)	Stem	100
DAE of Haldi (*Curcuma longa*)	Rhizomes	100
DAE of Tulsi (*Ocimum tenuiflorum*)	Leaf	100
DAE of Amla (*Phyllanthus emblica*)	Fruit	100
DAE of Mulethi / Yashtimadhu (*Glycyrrhiza glabra*)	Stem	100
Excipients : Quantity Sufficient		q.s.
Total		500
Preservatives : Sodium Propyl Paraben; Sodium methyl paraben;		

Table 4: SPMs of Immunoboost Herbal Extract Capsule (IBHEC).

S. No.	**Constituent**	**Source**	Uses
1.	Jatrorrhizine	*Tinospora cordifolia* Linn.(Giloy)	Palmatine; insulin mimicking effect; Jatrorrhizine; mimicking effect; Magnoflorine; Insulin release
2.	Berberine		
3.	Palmatine		
4.	Magnoflorine		
5.	Jateorine		
6.	Curcumin	*Curcuma longa* (Haldi) Zingiberaceae	Anti-inflammatory, Antioxidant, Liver toxicity, Skin disorders, Respiratory tract diseases, Anti-microbial, Arthritis, Alzheimer's disease, Anti-aging, Immunobooster, heartdiseases
7.	Curcuminoids		
8.	Eucalyptol		
9.	p-Coumaric		
10.	Zingiberene		
11.	Vanillic acid		
12.	Borneol		
13.	Sabinene		
14.	P-cymene		
15.	Eugenol	*Ocimum tenuiflorum* (Tulsi) (Family-Lamiaceae)	Immunno-stimulatory; Enhance secretion of phlegm (relieve cough); Anti-inflammatory; Anti- microbial; Adaptogenic; Improve Digestion; Reduce Stress; Antioxidant; Anti-microbial; Cure Respiratory diseases;
16.	methyl cinnamate		
17.	Linalool		
18.	β-elemene		
19.	Camphor		
20.	Linalyl acetate		
21.	ß- Ocimene		
22.	Eucalyptol		
23.	Neral		
24.	Geranyl acetate		
25.	Phyllantine	(*Phyllanthus emblica / Emblica officinalis*) Amla / Indian Gooseberry	Immunomodulatory, Analgesic, Antioxidant, Anti-inflammatory, Antidiabetic, Anticancer, Hypolipidaemic, Anti- HIV-1, Chemoprotective,
26	Phyllemblin		
27	Tannin (ellagitannins)		
28.	Vitamin C		
28	Kaempferol		
29.	Flavonoids		

30.	Polyphenols	(Euphorbiaceae; Phyllanthaceae)	Hepatoprotective, Cardioprotective.
31.	Amino acids		
32.	Diterpenes, triterpene		
33.	Gallic acid		
34.	Glycyrrhizin	*Glycyrrhiza glabra* (Mulethi) (Fabaceae) liquorice"	Antiviral, antimicrobial, anti-inflammatory, livertoxicity,
35.	licoricidin		
36.	glycyrrhizic acid		
37.	18β- Glycyrrhetinic acid		
38.	Licochalcone		
39.	Glabridin		
40.	Isoliquiritigenin		

The Immunoboost Herbal Extract Capsule (IBHEC) formulation contains extracts of 05 medicinal plants like Giloy Tulsi, Amla, Haldi, Mulethi as ingredients with 40 SPMs (Table 5).

Table 5: Comparison of IBT and IBHEC.

Parameter	Immunoboost Tablet (IBT)	Immunoboost Herbal Extract Capsule (IBHEC)
Constituents	94 SPMs from 12 Medicinal Plants / Herbs	40 SPMs from 05 Herbal Drugs
Pharmacologi-cal / MedicinalUses	Immunity Booster , anti-viral, Antioxidant, Edema, Anti- inflammatory, Antibacterial;	Immunomodulatory, Anti-inflammatory, anti-viral, Antibacterial, antifungal.
Dose	1 Tablet BID (500 mg; After meal)	1 Capsule BID (500 mg; After meal)
Toxicity Profile	▪ Safe dose: 3000 mg/kg bw ▪ Toxicity absent.	▪ Safe dose: 2000 mg/kg ▪ Toxicity absent.
Side Effects	Safe and no side effects.	No side effects

Qualitative Chemical Analysis of IBT and IBHEC for PPMs /SPMs.

Herbal Formulation Immunoboost Tablet (Nature Flip) and Immunoboost Herbal Extract Capsule. (Medinutrica) were used. Preliminary phytochemical screening for the detection of various was carried out by using standard procedures described by described by Harborne (1973); Trease and Evans (1985); Sofowora (1993); Khandelwal (2008); Kokate (2005). Phytochemical screening of IBT and IBHEC showed thepresence of various PPMs and SPMs as summarized in Table 8.

Table 6 : PPMs and SPMs present in IBT and IBHEC.

Tests performed for	**IBT**	**IBHEC**
Alkaloid	+	+
Cardiac Glycosides	+	+
Terpenoids	+	+
Steroids	+	+
Proteins & Amino acids	+	+
Saponins	+	+
Phytosterols	+	+
Flavanoids	+	+
Phenolic Compounds	+	+
Tannins	+	+
Fats	+	+
Reducing sugars	+	+
Carbohydrate	+	+

- = absent; + = present

Adaptogenic Activity of Immunoboost Tablet & Immunoboost Herbal Extract Capsule

Adaptogenic Activity

(i) Forced swimming endurance test

(ii) Chronic cold restraint stress

Forced swimming endurance test (Shakti *et al*., 2011)

Table 7: Grouping of Animals for forced swimming endurance test.

Groups	Treatment/Dose	Animals
I: Normal	Normal saline (0.1 ml/ 100g)	6
II: Standard	*Withania somnifera* (100 mg/kg)	6
III: Drug Treated	IBT (500 mg/kg) for 07 days	6
IV: Drug Treated	IBHEC (500 mg/kg) for 07 days	6
❖ Animals were subjected to swimming stress test on the 8th day and calculated swimming time (mean).		
❖ Rats were allowed to swim till get completely exhausted (animal started drowning at end point).		

Chronic Cold Restraint Stress Test

Table 8: Grouping of Animals for Chronic Cold Restraint Stress Test.

Groups	Treatment/Dose	Animals
Group-I: Normal	Normal saline (0.1 ml/ 100g)	6
Group-II: Standard	*Withania somnifera* (100 mg)	6
Group-III: Drug Treated	IBT (500 mg) for 07 days	6
Group-IV: Drug Treated	IBHEC (500 mg) for 07 days	6
❖ Chronic Cold Stress was induced by exposing animals to 4ºC for two hours.		
❖ Animals were taken out and individually placed in separate containers (withpartition).		
❖ Containers were placed inside refrigerator and animals were exposed at 4ºC fortwo hours then animals were transferred back to their home cages.		
❖ Procedure was continued for 10 days.		

Swimming time was found to be 26.32 (I), 45.84 (II), 41.62 (III), 36.56 (IV) mins. Swimming time of group group I was significant (P< 0.01) when compared with group II, III, IV. (Table 4.20). In cold restraint stress model pretreatment with *Withania somnifera*, IBTand IBHEC were significantly reduces blood cell counts except lymphocytes.

Table 9: Mean swimming time.

Groups	Treatment/Dose	Mean Swimming Time (in min)
Group-I	Normal saline (0.1 ml/ 100g, p.o.),	26.32 ± 2.883
Group-II	*Withania somnifera* (100 mg/kg, p.o.)	45.84 ± 1.014**
Group-III	IBT (500 mg/kg, p.o.) for 07 days	41.62 ± 0.760**
Group-IV	IBHEC (500 mg/kg, p.o.) for 07 days	36.56 ± 1.333**

Values are Mean ± SEM (6 animals) One-way ANOVA. **P<0.01.

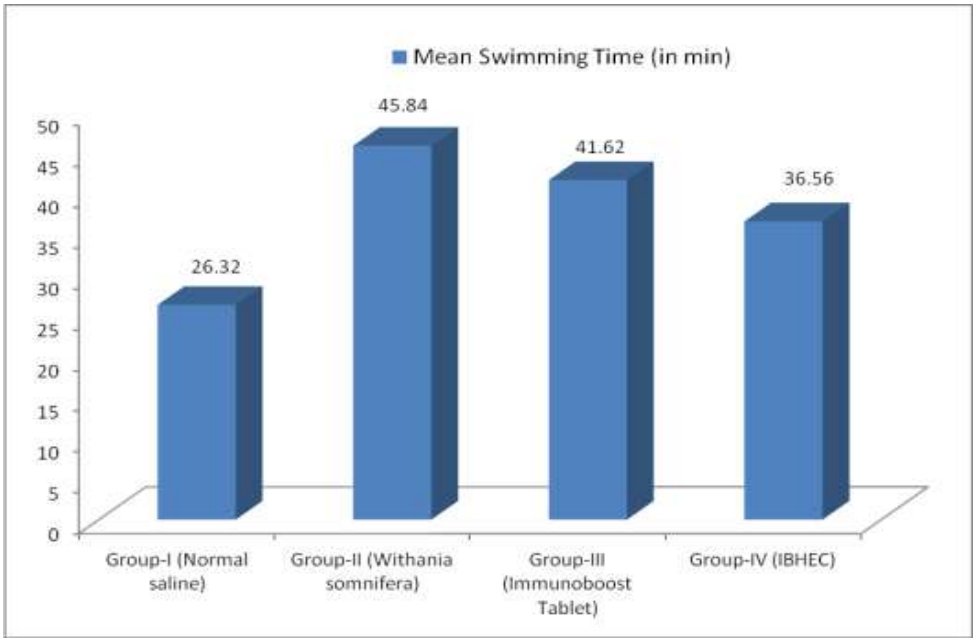

Figure 14: Mean swimming time in forced swimming endurance test.

***In-vivo* Immunomodulatory Activity of IBT and IBHEC**

DTH response

Table 10: Effect of IBT and IBC on DTH response.

S. No.	Groups	Paw volume (mm)			
		24 Hrs	48 Hrs	72 Hrs	96 Hrs
1.	I (Control)	1.44±0.028	0.76±0.020	0.44±0.016	0.17±0.014
2.	II (IBT: 500)	1.58±0.018	0.96±0.020	0.58±0.014	0.26±0.012**
3.	III (IBC: 500)	1.48±0.018	0.90±0.022	0.56±0.026	0.21±0.018*
4.	IV (Standard -50 mg/kg)	1.53±0.012	98.14±0.02	0.66±0.026	0.34±0.012***

Note: n= 6. Tabulation values represents mean ± SD (*P<0.05, **P<0.025, ***P<0.001

Humoral antibody (HA) titre

Table 11 : Effect of IBT and IBC on HA titre.

S. No.	Group	HA titre
1	Group-1 (Control)	11±1.022
2	Group-II (IBT:500 mg)	414±1.52**
3	Group-III (IBC:500 mg)	338.42±2.44*
4.	Group-IV (Levamisole 50 mg)	464± 2.62***

Note: n=6, mean ± SEM;

Total leukocyte count (TLC) Calculations

The area of the smallest = 1/16 mm3squareVolume of smallest square = 1/160 mm3 Total number of square counted = 16×4=64
Total number of cells counted = X
64/160 mm3 of diluted blood contains = X cells
So, 1 mm3 of diluted blood contains = 160/64 × X cells
1mm3 of undiluted blood contains = 160/64 × 20 × X cells.

The dose of IBC showed effect on TLC count compared to control group (Table 4.17).

Table 12 : Effect of IBT and IBC on Mean Leukocyte count.

Group	Leukocyte count (Mean; cu.mm)
1 (Control)	5.12×103 ± 0.2440
II (IBT:500 mg)	9.58×103 ± 0.3202**
III (IBC:500 mg)	6.94×103 ± 0.2262*
IV (Levamisole 50 mg)	15.08×103 ± 0.1484***

Note: n= 6, means ±SEM

Determination of total serum protein (Biuret Method)

Table 13: Effect of IBT and IBC.

Group	Total serum protein (g/100 ml)
I (Control)	7.4±0.1401
II (IBT:500 mg)	10.12±0.1112
III (IBC:500 mg)	8.8±0.962
IV (Levamisole 50 mg)	15.4±0.0240

Note: n= 6, means ± SD

Anti-inflammatory Activities of IBT and IBHEC

Carrageenan Induced Rat Paw Edema In Albino Wistar Rats

Animal study was approved with Form B - IEC/IAEC/ 2023/06; 17-02-2023

Table 14: Effect of IBT and IBHEC

Group	Paw edema (ml) Volume				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
I : Normal	--	--	--	--	--
II (Carrageenan: 100 mg/kg i.p.)	1.74±0.14	1.82±0.08	1.88±0.082	1.96±0.054	1.92±0.071
III : IBT (500 mg)	1.54*±0.06	1.40*±0.05	1.52*±0.07	1.44*±0.07	1.30*±0.10
IV : IBHEC (500 mg)	1.68±0.05	1.46*±0.06	1.52*±0.06	1.44*±0.18	1.40*±0.02
V : Standard (10 mg/kg)	1.50*±0.09	1.40*±0.04	1.44*±0.066	1.34*±0.22	1.28*±0.12

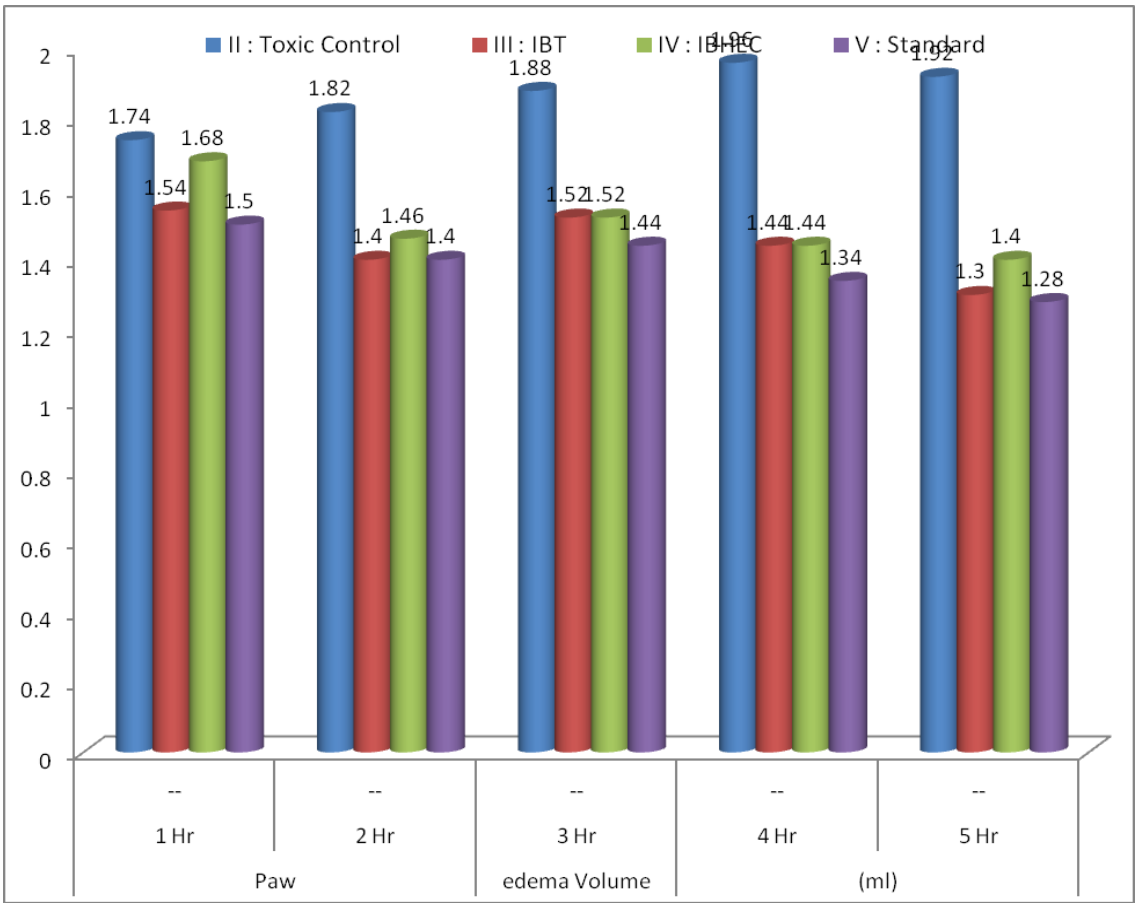

Figure 15: Effect of IBT and IBHEC.

Table 15: Inhibition (%) by IBT and IBHEC.

Group	% Inhibition				
	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr
III (IBT)	5.74	18.99	16.48	22.58	25.92

IV (IBHEC)	10.34	20.6	15.93	21.5	31.21
V (Indomethacin)	12.78	21.44	19.36	26.78	35.44

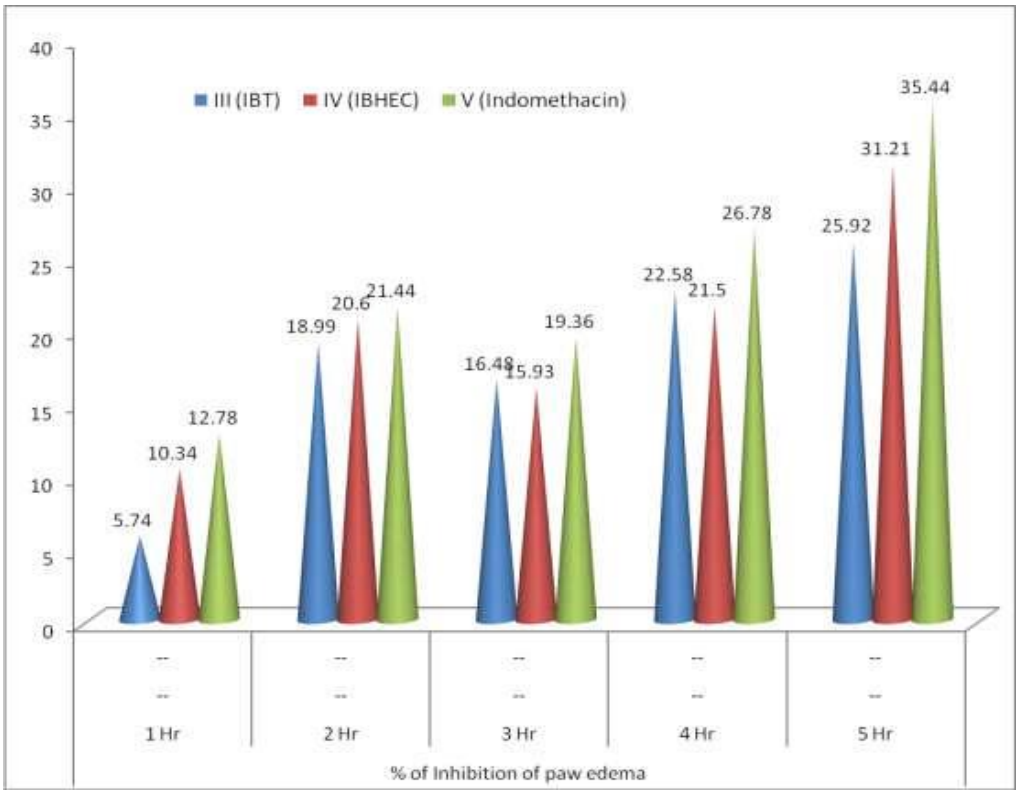

Figure 16 : Inhibition of paw edema by IBT and IBHEC

Results and Discussions

On comparative pharmacognostical assessment, it was found that Immunoboost Tablet (IBT; 500 mg Tablet; NatureFlip) contains 94 phytotherapeutic agents from dried aqueous extract or powder of 12 medicinal herbs / plants ingredients like Tulsi, Haldi, Guduchi, Haritaki, Baheda, Yastimadhu / Mulethi, Amla, Chitrak, Pippali, Kali Mirch, Sounth and Manjishta (Table 1-2).

Immunoboost Herbal Extract Capsule (IBHEC; 500 mg Tablet; Medinutrica) contains 44 phytotherapeutic agents derived from dried aqueous extract of ingredients as Tulsi, Amla, Mulethi, Giloy & Haldi (05 herbal drugs). (Table 3-4).

Further, it was found that IBT is marketed as an Antioxidant, Immunity Booster/ Immunomodulator, Cure inflammation and Edema (Anti-inflammatory), Inhibit microbial growth (Antibacterial, antifungal, anti-viral). On the contrary, IBHEC used as Immunomodulatory, Antibacterial, and Anti-inflammatory (Table 5).

Both phyto-formulations formulations IBT (500 mg Tablet) and IBHEC (500 mg capsule) are found to be safe (free from toxicity / side effects) and prescribed dose is 500 mg two times daily (after meal). IBT (NatureFlip) formulation was found to be more powerful immunomodulatory herbal formulation (94 SPMs from 12 medicinal herbs) then IBHEC (Medinutrica) immunomodulatory formulation (44 SPMs from 05 herbal ingredients).

Phytochemical studies of IBT and IBHEC showed presence of alkaloids, glycosides, polyphenols / phenolics, flavonoids, steroids, saponins and terpenoids (Table 6). Higher concentrations of both TPC and TFC (antioxidant activity is directly proportional to the amount of TPC and TFC) were found in IBT (TPC: 1226.24 ± 5.12 mg GAE/100 gDW; TFC : 9.7 mg of Quercetin eq./100 mg crude extract) then IBHEC (TPC: 684.16 ± 4.32 (mg GAE/100 gDW; TFC: 7.3 mg of quercetin equivalent/100 mg crude extract) because of high concentration of phenolic and flavonoid nature SPMs in Immunoboost Tablet, NatureFlip.

Further, both the formulations (IBT and IBHEC) induced significant free radical-scavenging (NO) activity and their antioxidant activities were increased with increased concentrations (regression equations significant at $p < 0.05$). IBT was found to produce significant NO scavenging activity (IC50 value 97.10 µg/ml). Antioxidant activity of IBT was significant (data were presented as means±S.D.) and one way analysis of variance (ANOVA) was also performed.

In adaptogenic activity, swimming time was found to be 26.32 (I), 45.84 (II), 41.62 (III), 36.56 (IV) mins. Swimming time of group group I was significant ($P< 0.01$) when compared with drug treated group II (*Withania somnifera*), III (IBT), IV (IBHEC). Besides, in cold restraint stress model pretreatment with *Withania somnifera*, IBT and IBHEC have slightly reduces blood cell counts (except lymphocytes) (Table 7-9; Figure 14).

Administration of herbal formulations IBT and IBC and Levamisole (standard / reference) showed increase in HA titre values when compared to control group (Table 11). IBT and IBC (500 mg/kg each) induced powerful RRBC membrane stabilizing properties and also produced significant anti-inflammatory effects (decrease in edema) when compared with Indomethacin (standard drug) (Table 14-15; Figure 15-16).

Conclusions

On the basis of literature review of ingredients of 500 mg TabletImmunoboost Tablet (IBT; NatureFlip) it was found that 94 SPMs were present in dried aqueous extract / powder made from 12 plants like Amla, Haldi, Kali Mirch, Baheda, Guduchi, Haritaki, Tulsi, Chitrak, Pippali, Yastimadhu / Mulethi, Sounth and Manjishta. Besides, 500 mg Capsule of Immunoboost Herbal Extract Capsule (IBHEC; Medinutrica) derived from dried aqueous extract of 05 herbal drugs ingredients (Amla, Tulsi, Giloy, Haldi and Mulethi) showed presence of 44 SPMs**.** IBT formulation was found to be more powerful immunomodulatory herbal formulation (12 medicinal herbs; 94 SPMs) then IBHEC formulation (05 herbal ingredients; 44 SPMs) and both phytoformulations were found free from toxicity / side effects (safe / non-toxic with wide safety margin. Further, IBT and IBHEC showed presence all category of PPMs and SPMs and higher content of TPC and TFC were found in IBT then IBHEC because of high phenolic and flavonoid content. In safety and toxicity evaluation studies, no mortality was observed even at the dose of 2000 mg/kg. body weight (non-toxic constituent / wide safety margin). Both IBT and IBHEC showed good adaptogenic properties (anti-stress). IBT and IBC (500 mg/kg each) induced powerful RRBC membrane stabilizing properties and also produced significant anti-inflammatory effects (decrease in edema) when compared with Indomethacin (standard).

REFERENCES:

1. Acharya, D., Shrivastava, A. (2008). *Indigenous Herbal Medicines:Tribal Formulations and Traditional Herbal*. 1st ed. Jaipur. Aavishkar Publishers. pp.1-45.
2. Agarwal, S.S., Singh, V.K. (1999). *Proc Indian Natl Sci Acad*. 65, pp. 179-204.
3. Ahmed, W., Galati, J., Christos, P.J., Longman, R., Scherl, E. (2021). *Clinical Gastroenterology and Hepatology*. pp. 34.
4. Alfons, B., Patrick, M. (2001). *J Leukoc Biol.* 70, pp. 849-860.
5. Alamgir, M., Uddin, S.J. (2010). *In: Ethnomedicine.* Research Signpost,pp. 217-224.
6. El-Sheikh, A.L.K. (2008). *Renal transport and drug interactions of immunesuppressants*. Netherlands: Radbound University. pp. 62.
7. Harborne, J. B. (1973). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. Springer Publications. pp. 1-288.
8. Jaramillo, M.,Alejandra, M. (2001). *Amer. J. of Botany*. 88(4), pp. 706–716.
9. Juyal, P.D., Singla, L.D. (2007). *Herbal immunomodulatory and therapeutic approaches to control parasitic infection*. pp. 311-315.
10. Khalander, S.D., Naga, A., Jilani, B.M. (2018). *International journal of pharmaceutical, chemical and biological sciences*. pp. 1-12.
11. Khandewal, K.R. (2008). *Prac. Pharmacognosy*. Nirali Prakashan,edition: 19., pp. 1-67.
12. Kokate, C.K. (2005). *Practical pharmacognosy*. 5th Edition, VallabhPrakashan: New Delhi. pp.107- 111.
13. Premanathan, M., Rajendran, S., Kathiresan, K. (2000). *Indian J. Med.Res*. 112, pp. 73-77.
14. Shakti, P., Jiban, D., Amit, M. (2011). *Int. Jour. of Drug Dev. & Res.* 3(1), pp. 84-96.
15. Shi, J., Jui-Hsia, W., Timothy, J M. (2021). *Biochemistry and ChemicalBiology*. 10, pp.1-16.
16. Sofowora, A. J. (1993). *Altern. Complement. Med.* 2 (3), pp. 365-372.
17. Thatte, U.M. and Dahanukar, S.A.(1997). *Immunomodulation*.Upadhyay SN, Narosa Publishing House. pp. 41-148.
18. Trease, G.E., Evans, W.C. (1985). Pharmacognosy. 17th edn., BahivTinal, London: pp, 149.
19. Wagner, H. (1984). Economic and Med. Plant Res. In: Hikino H, Farnsworth, N.R., Ed. 1. London. Academic Press. pp. 113-153.
20. Xiu-feng, Y.C., Jia-jun, H., Zhou, N., Yang, L. (2007). *Bioorg MedChem Lett*. 17(9), pp. 2408-2413.
21. Zarrin, A.A., Bao, K., Lupardus, P., Vucic, D. (2021). *Nature Reviews :Drug Discovery.* 20, pp. 39–63.