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EVALUATION OF IN-VIVO & IN-VITRO METHODS USING IMMUNO-MODULATORY ACTIVITY OF BRASSICA OLERACEA L. VAR. ITALICA PLENK

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ABSTRACT

The present study was aimed to investigate Immuno-modulatory activity of *Brassica Oleracea* L. var. *italica* Plenk. The assessment of immuno-modulatory activity was carried out by using Neutrophil Adhesion Test (In-Vivo Method) & T-Cell Population (In-Vitro Method). Oral administration of extract of *Brassica Oleracea* L. var. *italica* Plenk. Significantly showed Immuno-modulatory activity increases percentage neutrophil adhesion, increases lymphocytes & No. rosette count when result compared with control group. In conclusion, ethanolic & aqueous extract of *Brassica Oleracea* L. var. *italica* Plenk. showed presence of flavonoids, phenolic compound & vitamin C. But aqueous extract showed maximum immunomodulatory activity by both In-vivo & In-vitro methods.

KEYWORDS: Immuno-modulatory activity, *Brassica Oleracea* L. var. *italica* Plenk, Neutrophil Adhesion Test, T-Cell Population, Immunostimulant's.

1. INTRODUCTION

The primary health care herbal medicines are still the pillars of about 75 to 80% of theworld's populace, generally in the emergent nations. This is mainly due to the widespread belief that herbal medicines have no side effects as well as being cheap and readily available. According to the World Health Organisation (WHO), the use of herbal medicines worldwide exceeds that of conventional medicines by 2-3 times. The uses of plants for treatment purposes predate human history and form the origin of much of modernist medicine. One hundred years ago, most of the few active drugs / many conventional drugs obtained from plant

sources. Examples include aspirin (from the willow bark), digoxin (from the foxglove), quinine (from the cinchona bark), morphine (from the opium poppy/ breadseed poppy),[1] paclitaxel/ docataxel (from the bark of the pacific yew tree), podophyllotoxin (from the plant of podophyllum), vincristine/ vinblastine (from the vinca plant), curcumin (from the turmeric plant), etoposide/teniposide (from the podophyllum peltatum), etc.[2]

Immunomodulation

Immunomodulation is defined as the alteration of the immune response that can increase or decrease the immune response. Immunomodulators are generally two types based on their effects immunostimulant's & immunosuppressant's. Increases the immune responsiveness is called immunostimulation. Decreases the immune responsiveness is called immunosuppression.[8] Potential uses of immuno-modulators in clinical drugs include reconstitution of immunodeficiency (e.g., treatment of AIDS) & suppression of extreme immune function (e.g., treatment of graft's rejection or auto-immune disease).[3]

The immune system is designed to prevent the hosts from invading pathogens and to eliminate disease. The human body is occurring two types of immune response:

I) Innate immune response: The innate immune response is the first line of the defensemechanism against physical, biochemical and cellular components.

II) Adaptive immune response:

a) Humoral immunity - Antibody production - killing extracellular organisms.

b) Cell-mediated immunity - cytotoxic / killer T cells - that kill viruses and tumor cells.[4]

Immunomodulators are natural or synthesized substances that can control the immune system's equilibrium; these may be immuno-stimulant or immuno-suppressant. The function of the immune response is very important for the prevention and recovery from infectious diseases. Activation of the immune response contributes to decreases the risk of chronic disease. Some food & plant intake modulates as well as activates immune function. People are becoming more interested in using herbal medicines as agents that can modify the immune system and prevent infection. Groups of compounds such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids, tannins and glycosides have been reported to be responsible for the immunomodulatory activity of plants.[5]

Herbal drugs are easily affordable and less potent than synthetic prescription immuno-modulators but are also less likely to cause side effects. Therefore, there is a needed to search

for plants with immuno-modulatory activity to offer novel strategies for the treatment of infectious disease.

In Covid-19 situation immunity is important criteria for human being because weak immunity is causes corona virus infection. So enhancement of immune responsive is more important to prevent corona virus infection.

For example *Capparis zeylanica, Nelumbo nucifera, Allium sativum, Boerhaavia diffussa, Rhododendron spiciferum, Aloe vera, Asparagus racemosus,* etc.[4]

Future Scope

As immune system is known to be involves in the etiology as well as pathologic mechanisms of many diseases it has tremendously increased the need of drugs which are effective on immune system. Synthetic immunomodulators have drawbacks, so there is a need of development of herbal immunomodulators. This study will help the researchers to find out the new plants with immunomodulatory activity based on the parts used and theirchemical constituents.[6]

1) *Brassica Oleracea* L. var. *italica* Plenk

Botanical Name: - *Brassica Oleracea* L. var. italica Plenk. Family : - Brassicaceae

Common Name: - Broccoli.

Fig. No: 1. *Brassica Oleracea* L. var. *italica* Plenk.

❖ **Vernacular Names in India**

Table. No 1: Vernacular Name.

English	**Broccoli**
Marathi	Hiravi Kobi
Hindi	Hari Phool Gobi
Kannada	Kosugedde
Gujarati	Hari Phool Gobi

❖ **Occurrence and Description of Plant**

- **Geographical Distribution**

Brassicaceae family is southwestern and central Asia & the Mediterranean region whereas the arctic, western North America & the mountains of South America are secondary centers of variegation/ diversity.[7]

Broccoli is native to Mediterranean region. Broccoli is a cultivar of wild cabbage. Wild cabbages originate along the northern and western coasts of the Mediterranean, where it was apparently domesticated 1000 years ago. The family contains species of great economic importance, providing much of the world's winter vegetables.[8]

- **Plant Description**

The leaves are alternate (rarely opposite), sometimes arranged in basal rosettes; in the rare shrubby cruciferous of the Mediterranean, its leaves are found mainly in terminal rosettes and can be leathery and evergreen. Very often they are incised with pinnacles and don't have stipules.[7]

❖ **Chemical composition**

Broccoli is high in vitamins C, K, and A, as well as dietary fiber; it also contains multiple nutrients with potent anti-cancer properties, such as diindolylmethane and small amounts of selenium.[9]

2. MATERIAL AND METHODS

i) Drugs, chemicals & solvents: (Analytical grade drugs & chemicals are used.

All the drugs chemicals were analytical grade. Drugs & chemicals used in this experimentation as follows chloroform**,** ethanol, pet ether (40-60), ethyl acetate, methanol, diethyl ether, benzene, silica for TLC, silica for column, Levamisole, EDTA, Alsever's solution.

ii) Collection of plant material

The fresh flowering stalks of *Brassica Oleracea* L. var. *italica* Plenk. was collected in the month of November 2020 from the vegetable market (Khandekar vegetable market) in Sangli region, Maharashtra.

iii) Authentification of plant

The plant was authenticated by Mr. M. D. Wadmare, H. O. D. of Botany Department, Smt. K. W. C. Sangli.

iv) Drying of plant material

In the current study the collected flowering stalk was sorted carefully & washed thoroughly to remove dirt & debris. The plant material was cutting & spread out in thin layer on drying trays, kept in shade for 30 days. The drying trays were placed at a sufficient height above the ground to ensure proper air circulation and consistent drying of plant material. & avoid mould formation. After complete drying of flowering stalk was powdered by mixer grinder to obtain coarse powder.

v) Extraction of plant material

Aqueous Extraction

Chloroform water was prepared as 10% chloroform & 90% water (10:90). Then take 150-200gm of dry powder of *Brassica Oleracea* L. var. *italica* Plenk. was added in one liter of chloroform water I.P. (10%) contained in a round bottom flask. The flask was plugged with muslin cloth & kept at room temperature. It was shaken periodically up to seven days. Then it was filtered, mark was pressed & filtrate was collected. The extract was stored in a bottle in refrigerator at 4^{o}C.

Organic Solvent Extraction

Organic solvents extraction is carried out by using Soxhlet extraction. Soxhlet extraction performed in PG Chemistry lab.

Fig. No: 2. Soxhlet Extraction Apparatus.

Extraction was performed with organic solvents chloroform & ethanol (70%). About 100-150 gm of dry fruit powder of *Brassica Oleracea* L. var. *italica* Plenk. was extracted by chloroform by continuous Soxhlet extraction. The extraction was continued till the solvent became colorless. The chloroform extract was filtered & powder in the extraction apparatus is removed from extractor, dried & then it was used for extraction with ethanol. These chloroform & ethanol extract was stored in separate bottles & labeled.

vi) Phytochemical Investigation

Brassica Oleracea L. var. *italica* plenk. Three ethanolic & aqueous extracts was prepared, optimized & then to check phytochemical test.[10,11]

❖ **Animals**

The protocol used in this study for the use of rat as animal model for immunomodulatory activity was approved by Institutional Animal Ethical Committee (IAEC) of ABCP, Sangli. (Protocol No:-IAEC/ABCP/11/2020-21).

Swiss albino rat eight weeks old, either male/female, weight 150-200 gm were used for study. The animal care & handling was carried out according to CPCSEA guidelines. The animal study was performed in pharmacology research laboratory, ABCP, Sangli.

❖ **Acute Toxicity**

The acute toxicity of *Brassica Oleracea* L. var. *italica* plenk was reported. LD50 of aqueous & ethanol extracts of flowering stalk of *Brassica Oleracea* L. var. *italica* plenk was 4000mg/kg.[12]

❖ **Immunomodulatory Activity Methods**

A) In-Vivo Study

1) Neutrophil Adhesion Test

➢ **Procedure**

- In this test animals are divided into 6 groups comprising 5 animals in each group.
- Group I was kept as a control & received vehicle only water (10 ml/kg).
- Group II was kept as a standard & received standard drug levamisole (50 mg/kg).
- Group III was kept as a test-III & received the EBO sample no-I.
- Group IV was kept as a test-IV & received the ABO sample no-II.

Table No. 2. Group & Treatment Schedule for Neutrophil Adhesion Test.

Sr. No.	Groups	Treatment	Dose
1	Group I	Control (water)	10ml/kg
2	Group II	Standard (Levamisole)	50mg/kg
3	Group III	Ethanolic *Brassica Oleracea* L. var. *italica* Plenk.	0.12ml
4	Group IV	Aqueous *Brassica Oleracea* L. var. *italica* Plenk.	0.12ml

- On 16th day of the treatment blood sample from the entire group was collected by puncturing retro-orbital plexus under mild anesthesia.
- Blood was collected in vials pretreated by disodium EDTA & analyzed for Total Leukocyte Count (TLC) & Differential Leukocyte Count (DLC).
- After initial count blood sample was collected with nylon fiber (80mg/ml, previously sterilized by alcohol) for 15 min at 37^{o} C & the incubated drug sample was analyzed for TLC & DLC.
- The product of TLC & % neutrophils adhesion was calculated as follows.[13]

$$\% \text{ Neutrophil adhesion} = \frac{NIU - NIT}{NI_U} \times 100$$

Where,

NI_U - Neutrophil index before incubation with nylon fiber. NI_T - Neutrophil index after incubation with nylon fiber.

B) In-Vitro Study

1) T-Cell Population

➢ **Procedure**

- On 0th day, all groups were sensitized with 0.1 ml of SRBC containing 1×108 cells,i.p.
- Animals were divided into different groups each containing 5 animals.
- Group I was kept as a control & received vehicle only saline (10 ml/kg).
- Group II was kept as a standard & received standard drug levamisole (50 mg/kg).
- Group III was kept as a test-I & received the EBO sample no-I.
- Group VI was kept as a test-II & received the ABO sample no-II.

Table No. 3: Group & Treatment Schedule for T-Cell Population Test.

Sr. No.	Groups	Treatment	Dose
1	Group I	Control (water)	10ml/kg
2	Group II	Standard (Levamisole)	50mg/kg
3.	Group III	Ethanolic *Brassica Oleracea* L. var. *italica* Plenk.	0.12ml
4.	Group IV	Aqueous *Brassica Oleracea* L. var. *italica* Plenk.	0.12ml

- On 11th day, blood was collected from the retro-orbital plexus & anticoagulanted with Alsever's solution in separate test tube.
- Test tube containing blood was kept in sloping position (45^{o}) at 37^{o} c for 1 hour. RBC were allowed to settle at bottom & supernatant was collected from each test tube by using micro-pipette contains lymphocytes.
- 50 µl of lymphocyte suspension & 50 µl SRBC were mixed in test tube & incubated.
- Resultant suspension was centrifuged at 200 rpms for 5 min & kept in a refrigerator at4^{o} c for 2 hrs.
- The supernatant fluid was removed & one drop of cell suspension was placed on a glass slide.
- Total lymphocytes were counted & a lymphocyte binding with three or more erythrocytes was considered as rosette & no. of rosette was counted.[14]

Table no. 4: Composition of Alsever's Solution.

Chemicals	Quantity (g/L)
Sodium chloride	4.2 gm
Sodium citrate	8.0 gm
Citric acid anhydrous	0.55 gm
Glucose	20.5gm
Distilled water q.s.	1000ml

3. RESULTS AND DISCUSSION

Phytochemical Investigation

Table No. 5: Results of Phytochemical Investigation.

A. Test for Flavonoid

Sr.No.	Chemical Test	Observation	Inferences	
			Brassica Oleracea L. var. *italica* plenk.	
			Ethanol	Aqueous
1.	**Alkaline Test:** Plant extract + 10 % ammonium hydroxidesolution	Yellow Fluorescence	**+ ve**	**+ ve**
2.	**Lead Acetate Test:** Plant extract + 10% leadacetate sol^{n} (few drops)	Yellow Precipitate	**+ ve**	**+ ve**
3.	**Conc. Sulphuric AcidTest:** Plant extract + Conc. H2SO4	OrangeColour	**+ ve**	**+ ve**
4.	**Shibata's Test:** Plant extract + dissolve in 1-2 ml 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl.	Red colour	**- ve**	**+ ve**

B. Test for Phenolic Compound

1.	**Iodine test:** 1mL extract + few drops ofdil. Iodine sol.	A transient red colour	**+ ve**	**+ ve**
2.	**Gelatin test:** Plant extract is dissolved in5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	**+ ve**	**+ ve**
3.	**Lead acetate test:** Plant extract is dissolved in 5mL distilled water + 3mLof 10% lead acetate sol^{n}.	A white precipitate	**+ ve**	**+ ve**
4.	**Potassium dichromatetest:** Plant extract + few dropsof potassium dichromate solution	A dark colour	**+ ve**	**- ve**

C. Test for Alkaloids

1.	**Dragendroff's test:** Few ml filtrate + 1-2 ml *Dragendroff's reagents*	A reddish-brown precipitate	**+ ve**	**+ ve**
2.	**Wagner's test :** Few ml filtrate + 1-2 drops of *Wagner's reagent* (Alongthe sides of test tube)	A brown/reddish precipitate	**- ve**	**+ ve**

D. Test for Cardiac Glycosides

1.	**Keller-Killani test :** 1mL filtrate + 1.5mL glacial acetic acid + 1 drop of 5% ferricchloride + conc. H_2SO_4 (along the side of test tube)	A blue coloured solution (in acetic acid layer)	**+ve**	**-ve**
2.	**Test for Cardenolides :** Extract + pyridine + Sodium nitroprusside +20% NaOH	A red colour, fades to brownish yellow	**+ve**	**+ve**
3.	**Baljet test :** 2mL extract + a drop of *Baljet's reagent*	A yellow-orange colour	**+ve**	**-ve**

E. Test for Vitamin C:

1.	Add 2ml of a 2% w/v solution + few ml of 2,6- dichlorophenolindophenol solution	Solution is decolorized	**+ ve**	**+ ve**
2.	Add 2 ml of 2% w/v solution + 2ml of water + 0.1 gm of sodium bicarbonate + 20 mg of ferrous sulphate shake & allow to stand. After that add 5ml of 1M H_2SO_4.	Deep violet Colour isproduced after add H_2SO_4 colour will disappears.	**+ ve**	**+ ve**

DISCUSSION

The presence of flavonoids, phenolic compounds, vitamin C, alkaloids & Cardiac glycosides major constituents in ethanolic &aqueous extract of *Brassica Oleracea* L. var. *italica* plenk.

Pharmacological Screenings

In-Vivo Method

Result of Neutrophil Adhesion Test

Effect of *Brassica Oleracea* L. var. *italica* plenk on Neutrophil Adhesion Test.

Table No. 6: Neutrophil Adhesion Test Observation.

Animals	% Neutrophil Adhesion			
	Control	**Standard**	**EBO**	**ABO**
1.	26.48%	66%	54.16%	59.23%
2.	31.42%	70.48%	48.64%	60.43%
3.	24.78%	65.17%	52.36%	61.42%
4.	26.47%	66.29%	54.61%	57.26%
5.	27.12%	68%	59.105	59.30%

❖ ***Brassica Oleracea* L. var. *italica* plenk**

Values are expressed as (Mean ± S.E.M) n=4**** P<0.0001 statistically significant when

compared with control group by ANOVA followed by Dunnett test. The result is as follows:

Table No. 7: Results of % Neutrophil Adhesion.

Sr.No.	Group	% Neutrophil Adhesion
1.	Control	27.25 ±1.111
2.	Standard	67.19 ±0.943
3.	EBO	53.77 ±1.697
4.	ABO	59.53 ±0.6956

Effect of EBO & ABO extract on neutrophils activation by the neutrophil adhesiontest is shown in [Table: 11].

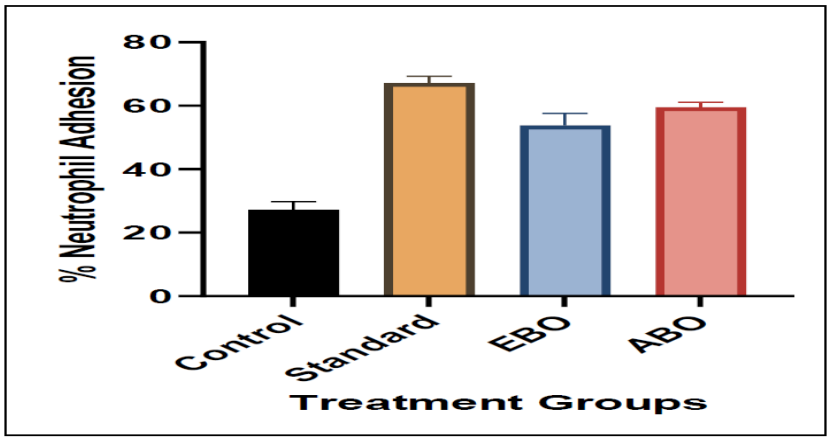

Fig: 3. Graphical Representation of Neutrophil Adhesion Test.

DISCUSSION

Above graphical representation observed that ABO showed highest % neutrophil adhesion (59.53 ±0.6956) than EBO (53.77 ±1.697). When compare with control group showed possible Immunostimulant effect.

In-Vitro Method

I] Results of T-Cell Population Test.

A] Effect of *Brassica Oleracea* L. var. *italica* plenk on T-Cell Population Test.

Table No. 8. T-Cell Population Test Observation.

Animals	% Lymphocyte Count				No. of Rosettes			
	Control	Standard	EBO	ABO	Control	Standard	EBO	ABO
1.	11%	19%	11%	14%	12	27	10	17
2.	9%	22%	10%	16%	8	24	8	21
3.	14%	21%	16%	19%	14	29	12	22
4.	14%	23%	16%	17%	13	22	14	18
5.	11%	18%	14%	15%	7	32	13	16

❖ ***Brassica Oleracea* L. var. *italica* plenk**

Values are expressed as (Mean ± S.E.M) n=4**** P<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test. The results as follows:

Table No. 9. Results of % Absolute Lymphocyte.

Sr.No.	Group	% absolute Lymphocyte
1.	Control	11.8 ±0.9695
2.	Standard	20.6 ±0.9274
3.	EBO	13.4 ±1.249
4.	ABO	16.2 ±0.8602

Effect of EBO & ABO extract on lymphocyte activation by the t-cell population test is shown in [Table: 16].

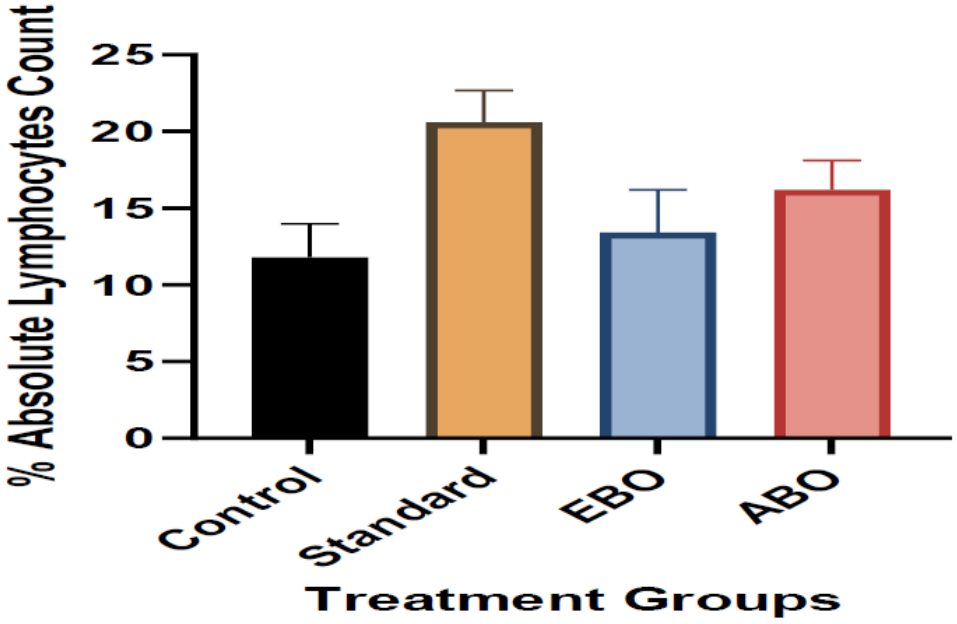

Fig: 4. Graphical Representation of T-Cell Population Test.

DISCUSSION

Above graphical representation observed that ABO showed highest % absolute lymphocyte count (16.2 ±0.8602) than EBO (13.4 ±1.249). When compare with control group showed possible Immunostimulant effect.

❖ ***Brassica Oleracea* L. var. *italica* plenk**

Values are expressed as (Mean ± S.E.M) n=4**** P<0.0001 statistically significant when compared with control group by ANOVA followed by Dunnett test. The results as follows.

Table No. 10: No. of Rosette Count.

Sr.No.	Group	No. of Rosette Count
1.	Control	10.8 ±1.393
2.	Standard	26.8 ±1.772
3.	EBO	11.4 ± 1.077
4.	ABO	18.8 ±1.158

Effect of EBO & ABO extract on lymphocyte activation by the t-cell population test is shown in [Table: 17].

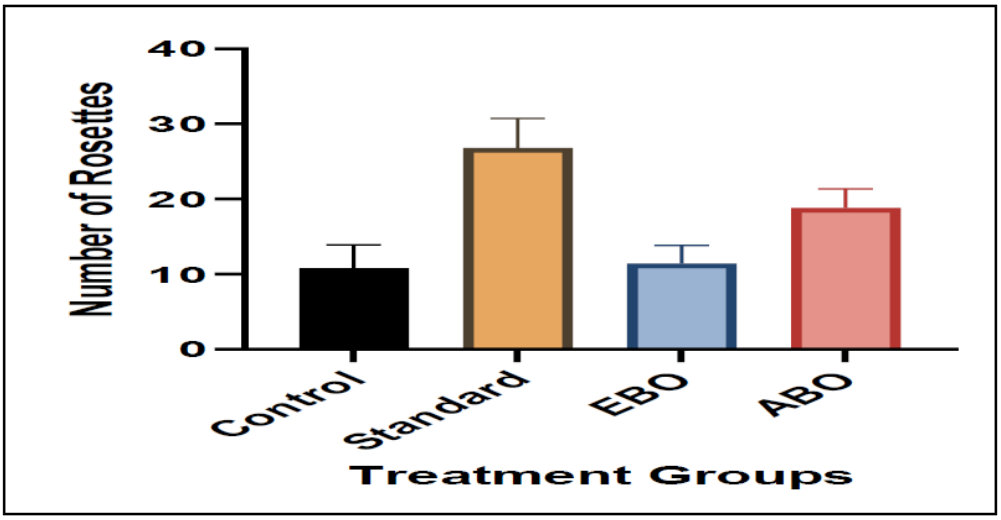

Fig 5: Graphical Representation of T-Cell Population Test.

DISCUSSION

Above graphical representation observed that ABO showed highest no. of rosette count (18.8 ±1.158) than EBO (11.4 ± 1.077). When compare with control group showed possible Immunostimulant effect.

4. CONCLUSION

Ethanolic & aqueous extract of *Brassica Oleracea* L. var. *italica* Plenk. showed presence of flavonoids, phenolic compound & vitamin c. But aqueous extract showed maximum immunomodulatory activity by both In-vivo & In-vitro methods.

5. REFERENCES

1. Pal SK, Shukla Y. Herbal medicine: current status and the future. Asian pacific journal of cancer prevention, 2003 Aug 20; 4(4): 281-288.
2. Ashraf MA. Phytochemicals as potential anticancer drugs: time to ponder nature's bounty. BioMed research international, 2020 Jan 31; 2020: 7.

3. Nagarathna PK, Reena K, Reddy S, Wesley J. Review on immunomodulation and immunomodulatory activity of some herbal plants. Int J Pharm Sci Rev Res, 2013 Sep; 22(1): 223-30.
4. Kumar SV, Kumar SP, Rupesh D, Nitin K. Immunomodulatory effects of some traditional medicinal plants. J Chem Pharm Res, 2011; 3(1): 675-84.
5. Sujono TA, Dian Kusumowati IT, Munawaroh RI. Immunomodulatory activity of Muntingia calabura L. fruits using carbon clearance assay and their total flavonoid and phenolic contents. Asian Journal of Pharmaceutical and Clinical Research, 2020; 13(2): 140-5.
6. Nawale R, Poojari S. Review on chemical constituents & parts of as Immunomodulators. Research Journal of Pharmaceutical, Biological & Chemical Sciences, 2013 March; 4(1): 76-89.
7. Anjum NA, Gill SS, Ahmad I, Pacheco M, Duarte AC, Umar S, Khan NA, Pereira ME. The plant family Brassicaceae: an introduction. In The plant family Brassicaceae 2012 (pp. 1-33). Springer, Dordrecht.
8. Kumar A, Choudhary AK, Rahi S. Scientific cultivation of Broccoli (Brassica Oleracea L. var. italica), 2014; 87-91.
9. Satwase AN, Pandhre GR, Kelapure NN, Aware BM and Thakare S. A Comprehensive Study on Antioxidant Activity and Antimicrobial Analysis of Broccoli (*Brassica oleracea)* Juice. International Journal of Pure & Applied Bioscience, 2018; 6(2): 1470-76.
10. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 2020; 8(2): 603-8.
11. Khandelwal K. Practical pharmacognosy. Pragati Books Pvt. Ltd, 2008 Sep 7.
12. Shah MA, Sarker MM, Gousuddin M. Toxicity Study of Brassica Oleracea Var. Italica Extracts in Sprague Dawley (SD) rats. International Journal of Pharmacognosy and Phytochemical, 2016; 8: 735-41.
13. Bagwan SA, Naikwade NS, Manure JY. Comparative Study of Immunomodulatory Activity of Marketed Ayurvedic Formulation. Indo American Journal of Pharmaceutical Sciences, 2017 Dec 1; 4(12): 4776-82.
14. Anarthe SJ, Sunitha D, Raju MG. Immunomodulatory activity for methanolic extract of Trigonella foenum graecum whole plant in wistar albino rats. American Journal of Phytomedicine and Clinical Therapeutics, 2014 Sep 30; 2(9): 1081-92.