WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 7, 986-994. Research Article ISSN 2277– 7105

EVALUATION OF IN-VITRO AND IN-VIVO IMMUNOMODULATORY ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACT OF *BIXA ORELLANA* (L.).

Pallavi Maruti Patil[1]*, Vanita G. Kanase[2] and Jignyasha Amit Kumar Raval[3]

[1]Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan-313003.

[2]Head of Dept. of Pharmacology, Oriental College of Pharmacy, Sanpada, Navi Mumbai Maharashtra-400705.

[3]Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan-313003.

Article Received on
04 May 2021,

Revised on 24 May 2021,
Accepted on 14 June 2021

DOI: 10.20959/wjpr20217-20800

***Corresponding Author**
Pallavi Maruti Patil
Research Scholar, Faculty of Science, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan-313003.

ABSTRACT

Objective: The aim of this study is to evaluate if *Bixa orellana* Linn. (Bixaceae) leaves have Immunomodulatory properties. **Methods:** Dried leaf powder was extracted in series of solvents, including Ethanolic Extract, chloroform, ethyl acetate, methanol, and water, and extracts were tested for Immunomodulatory effects in Swiss albino mice. Acute oral toxicity investigations have been completed, and animal has been shown to be alive for 24 hours at doses of up to 1000 mg/kg. Using plethysmometer, volume of hind paw is measured before and after inflammation is induced. Level of inflammation in untreated and test drug-treated mice was assessed. **Results:** Ethanolic Extract demonstrated most efficacies at dosage level of 250 mg/kg after 2 hours, according to findings. When compared to conventional medicine as vehicle and control for extract, if inflammation in treated animals is less than that in untreated animals, medicine is regarded to have Immunomodulatory action. Tail immersion method was used to assess analgesic efficacy of different extracts of *Bixa orellana* leaves. Results showed that methanolic extract (12.4 sec) had highest action at dosage of 500 mg/kg 120 minutes after delivery.

KEYWORDS:- *Bixa orellana* Linn, In-Vivo and In-Vitro, Ethanol Extracts, Immunomodulatory Activity.

INTRODUCTION

Immunomodulation is process of adjusting immune system's regulation.[1] It comes in both natural and man-made forms, and term may be used to describe following: immune system's self-regulation to modify immune responses to adaptive rather than maladaptive levels is known as homeostasis (using regulatory T cells, cell signaling molecules, and so forth) Immunomodulation is type of immunotherapy that involves inducing, amplifying, attenuating, or preventing immune responses to achieve therapeutic aims.[2] Immunomodulators are biological response modifiers that can strengthen or weaken immune responses. Immunomodulators have been used to treat malignancies, viral infections, autoimmune illnesses, and immunodeficiency illnesses in clinical practice. Immunomodulator-based immunotherapy is typically more successful when used in conjunction with or after chemotherapy and radiation treatment.[3,4]

Bixa orellana is a tiny evergreen tree native to Central, South, and North America.[5] Achiote or annatto is other names for it. Carotenoids bixin and norbixin, which are found in abundance in red seeds, are utilized to make annatto, commonly used red to yellow color. Dye is used to color clothing as well as variety of foods such as rice, butter, cheeses, soups, and soft beverages. Bixa leaves, decoctions (teas), and extracts have been used by indigenous peoples for many years as medical and folkloric treatments to cure headaches, dysentery, fever, different microbiological illnesses, heartburn, and indigestion, as well as astringent and to cure different skin disorders. Bixa leaf and root preparations are used to cure jaundice, diabetes, and hypertension in Trinidad and Tobago, according to ethnobotanical interviews. According to previously published scientific findings, plant has antivenom, antibacterial, anticonvulsant, analgesic, anti-diarrheal, enzyme producing, hypoglycemic, and antimutagenic properties. Plant is utilized as coloring ingredient in traditional Filipino cookery. It's also used to tint butter, margarine, cheese, drinks, and meat and fish. Bixa dye, often known as annatto dye, is made from outer layer of *Bixa orellana* seeds.[6]

Chemical constituents

Several research have looked into chemical contents that might be responsible for pharmacological actions of Bixa leaf extracts, and number of compounds with possible pharmacological actions have been discovered. Terpenes, triterpenes, steroids, saponins, cardiac glycosides, sugars, tannins, alkaloids, flavonoids, and other phenolic chemicals have been found in Bixa leaves in phytochemical screenings.[7] It's worth noting that in majority of

these investigations, general phytochemical screening procedures were used, with no particular chemical ingredients identified in any of these broad categories. Lack of information about chemical elements in Bixa leaves contrasts sharply with lack of information on chemical composition of Bixa seeds.[8] Bixin and norbixin, two carotenoids that are known to be key chemical elements of Bixa seeds (annatto), are responsible for yellow/orange/red hue of seed extracts. Furthermore, comprehensive investigation of Bixa seed extracts revealed presence of 107 chemicals, 56 of which were tentatively identified, and 51 of which were positively identified.[9]

MATERIALS AND METHODS

Collection of plant

The leaves of B. orellana were collected from naturally occurring tree in Kakatiya University's Biotechnology Department's Botanical Garden in Warangal, India. Identification and confirmation were done by trained taxonomist. Specimen was sent to institution's herbarium. Obtained plant material was cleaned of any extraneous organic components. In laboratory mixer, leaves were separated, shade dried, pulverized, and sieved.[10]

Preparation of extract

The weighed (70 g) leaf powder was subjected to series of Soxhlet extractions with Ethanolic Extract (60-80°C), chloroform, ethyl acetate, methanol, and water, each solvent accounting for 3/4th of round bottomed flask, each lasting 6 hours, and each solvent extracted once. In desiccators, resulting solvent extracts were concentrated and dried.[11]

Animals

Mahaveer Enterprises in Hyderabad provided healthy Swiss albino mice for study, which were of either sex, of same age, and weighed 150-180 g. They were fed conventional chow diet and had to drink plenty of water. Animals were kept in polypropylene cages with conventional environmental conditions (12 hours of light/12 hours of darkness; 25°C, 35-60% relative humidity). Animals were handled according to CPCSEA norms, and study was carried out after Institutional Animal Ethics Committee approved it.[12]

Acute oral Toxicity and Large-Scale behavioral investigation

The mice were starved overnight, separated into six groups (n=6), and orally administered escalating dosages of Ethanolic Extract, methanol, and aqueous extracts suspended in groundnut oil (250, 500, 750, 1000 mg/kg body weight). After receiving extracts, animals

were monitored for first 2 hours for gross behavioral changes, then every 30 minutes for following 4 hours, and finally every 24 hours for next 72 hours to determine percentage mortality. As per the OECD (Organization for Economic Cooperation and Development) guideline number 423.[13,21]

Preparation of HRBCs with Immunomodulatory Activity in In-Vitro (Human Red Blood Cells)

Blood was taken and centrifuged from healthy human volunteers. After that, sterile pipettes were used to properly pipette supernatant. Packed cells were resuspended in isosaline in equivalent amount and centrifuged. Operation was repeated 4 times until supernatants were clear. After that, 10% HRBC suspension was made with normal saline and stored at 4°C until use.[14]

Effects of plant extracts on human red blood cell system

The reaction mixture (4.5 ml) was made up of 2 ml hyposaline (0.25 percent w/v NaCl), 1 ml isosaline buffer solution, pH 7.4 (6.0 g TRIS, 5.8 gm NaCl, HCl to regulate pH, and water to make 1000 ml) and varying volumes of extract solution in isotonic buffer (concentration=100 mg/ml) to bring total volume to After that, 0.5 mL of 10% HRBC in normal saline was added. There were two controls carried out. One control used 1 ml of isosaline buffer instead of extract (control 1), while other used 1 ml of extract solution but no red blood cells (control 2). (Control 2). For 30 minutes, mixture was incubated at 56°C. Tubes were chilled for 20 minutes under running water. Combination was centrifuged, and supernatant's absorbance was measured at 560 nm. Formula was used to calculate percentage of membrane stabilization.[15]

Immunomodulatory activity in In-vivo

Young adult both male and female Swiss albino mice weighing 180-220 g, acclimatized to laboratory settings and fed normal laboratory mice diet and clean water, were employed. Mice were fasted for 12 hours previous to experiment, but were given access to drink throughout. Mice were placed into eight groups, each with six rodents. Animals were given extracts that were either control, standard, or test. Arachis oil was used to make control and standard samples. Mark was created immediately beyond tibiotarsal junction on both hind paws, such that every time paw is dipped in mercury column up to specified level, paw volume is maintained. Following administration of test and standard samples for 1 hour, 0.1 ml of 1% carrageenan suspension (in normal saline) was subcutaneously injected into dorsal

area of subplantar surface of mice hind paw with 26 G needle. Each mice initial paw volume was measured before medication was administered. Using plethysmometer, paw volumes were measured after 0.5, 1, 2, 3, and 4 hours. By subtracting starting paw volume from paw volumes at various time intervals, any change in paw volume of mice was calculated. Average edema value was obtained by adding averages of each group at various hours. Each group's edema inhibition percentage was determined in comparison to its control group.

(A - B) x 100/A = percentage inhibition

Where represents mean increase in paw volume in mice given control and B represents mean increase in paw volume in mice given test.[16]

Table 1: Animal divisions for *Bixa orellana* immunomodulatory efficacy.

Groups	Extract
Group I	Ethanolic Extract (250 mg/kg)
Group II	Ethanolic Extract (500 mg/kg)
Group III	Methanolic Extract (250 mg/kg)
Group IV	Methanolic Extract (500 mg/kg)
Group V	Aqueous Extract (250 mg/kg)
Group VI	Aqueous Extract (500 mg/kg)

RESULTS AND DISCUSSION

After 72 hours, all of mice in acute oral toxicity investigation survived. This means extracts were found to be safe at dosage levels investigated. Because all of animals survived dosage of 1000 mg/kg body weight, extracts' LD50 will be more than 1000 mg/kg body weight. During research period, no significant behavioral changes were found. All of extracts had slight sedative effect on animals when given to them.[17]

Table 2: Acute oral toxicity studies are listed.

Extracts	Groups	Dose(mg/kg)	No. of mice each group	After 4 hours	After 24 hours
Ethanolic Extract	I	250	6	6	6
	II	500	6	6	6
	III	750	6	6	6
	IV	1000	6	6	6
Methanol	I	250	6	6	6
	II	500	6	6	6
	III	750	6	6	6
	IV	1000	6	6	6

***Bixa orellana* leaf extracts have immunomodulatory activity**

In-Vitro

Membrane stabilization of HRBCs at doses of 3 and 5 mg/ml was used to test Immunomodulatory effects of all leaf extracts in In-vitro. Findings of this inquiry were presented. Findings of this study show that Ethanolic Extract has strong Immunomodulatory efficacy. It provided approximately 67.47 percent protection at 3 mg/ml and 37.3 percent protection at 5 mg/ml, demonstrating that action was dosage independent. When compared to Ethanolic Extract, methanolic and aqueous extracts exhibited less significant action.[18]

Table 3: Immunomodulatory efficacy in In-vitro.

Treatment	Dose mg/ml	%Protection
Standard	3	72.04
	5	75.01
Ethanolic Extract	3	67.47
	5	37.35
Methanol	3	62.3
	5	25.6
Aqueous	3	34.99
	5	10.67

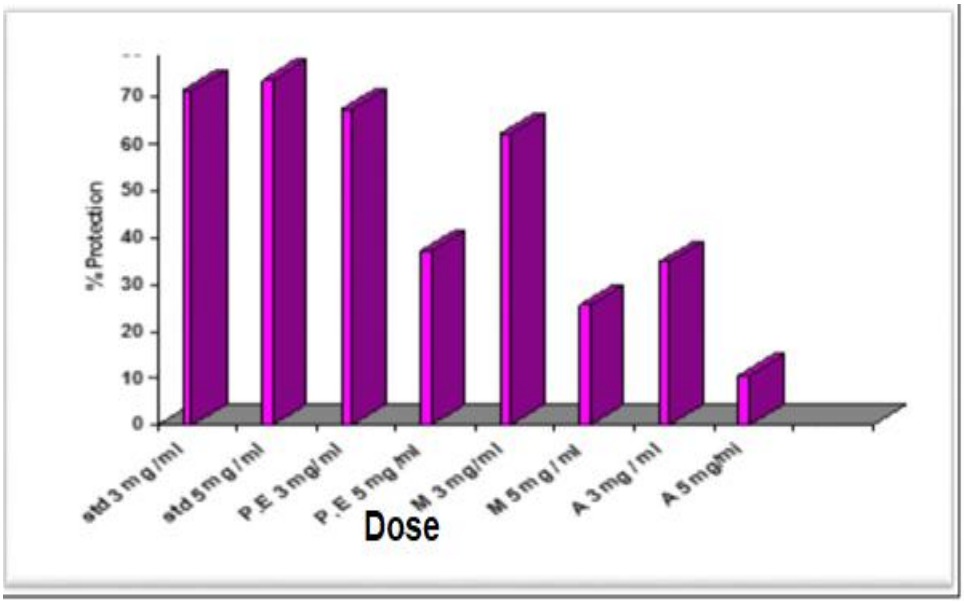

Figure 1: Percentage protection of *Bixa orellana* leaf extracts on HRBC membrane stability.

In- Vivo

All of leaf extracts were tested for Immunomodulatory activity in mice paw edema using carrageenan induced mice paw edema technique at doses of 250 and 500 mg/kg body weight.

These are findings of this inquiry. Findings reveal that all extracts had considerable proportion of protection against edema development, and that Immunomodulatory effect of extracts was not dosage dependent.[19]

Table 4: Immunomodulatory effect of *Bixa orellana* leaf extracts.

Name of drug	Dose (mg/kg)	*Mean Edema Volume (ml)				
		30 min	1 h	2 h	3 h	4 h
Control	-	0.23 ±0.02	0.34 ± 0.02	0.62 ± 0.05	0.75 ± 0.02	0.70 ± 0.03
Standard	100	0.15 ± 0.03	0.23 ± 0.03	0.31 ± 0.03	0.27 ± 0.04	0.34 ± 0.03
Ethanolic Extract	250	0.16 ± 0.02	0.19 ± 0.03	0.21 ± 0.05	0.28 ± 0.04	0.27 ± 0.04
	500	0.18 ± 0.02	0.22 ± 0.03	0.25 ± 0.02	0.31 ± 0.03	0.29 ± 0.03
Methanol	250	0.19 ±0.01	0.25 ± 0.03	0.28 ± 0.03	0.29 ± 0.05	0.28 ± 0.06
	500	0.21 ± 0.03	0.28 ± 0.04	0.38 ± 0.03	0.49 ± 0.04	0.38 ± 0.04

CONCLUSION

The in-vitro Immunomodulatory effect of Ethanolic Extract of *Bixa orellana* leaves was substantial, based on preceding findings. Ethanolic Extract, which was formed by carrageenan, had considerable in-vivo Immunomodulatory impact via reducing edema. However, activity was not similar to that triggered by typical medication in terms of quantitative activity. It's possible that this is related to usage of crude extracts. As result, isolating active principles will be beneficial in producing novel bioactive elements from these extracts that may have more activity. Aqueous, methanol, ethanol, and hydro alcohol extracts, as well as powdered leaf, were employed in investigations mentioned above. Extraction technique alters chemical composition and concentration of numerous elements. As result, pharmacological effects differ depending on solvent employed in extract extraction, resulting in chemical composition differences. Studies that show link between chemical makeup of extracts and their pharmacological effects are needed.[20]

ACKNOWLEDGEMENT

We authors would like to thank the authority, and acknowledge financial support for the research study provided by Mumbai University for granting Minor Research grant (Grant Number - APD/ICD/2019-20/762).

REFERENCES

1. Ahmed F, Khan RA. Study of Analgesic and Immunomodulatory activity from Plant extracts of Lactuca scariola and Artemisia absinthium. Pak J Islam Acad Sci, 1992; 5: 111-114.

2. Amos B, Adzu L, Binda CW, et al. Behavioral effects of aqueous extract of Guiera senegalensisi in mice and rats. Phytomedicine, 2001; 8: 356-361.
3. Aranez AT, Rubio RO. Genotoxicity of pigments from seeds of *Bixa orellana* (Astute) I determined by Allium test. Philipp J Sci, 1996; 125: 259-269.
4. Bruneton J. Pharmacognosy, Phytochemistry of Medicinal Plants, 1999; 2: 131-160.
5. Castello MC, Pathak A, Chandra N, et al. Indian J Exp Biol, 2002; 40: 1378-1381.
6. Dimo T, Fotio AL, Nguelefack TB, et al. Immunomodulatory activity of leaf extracts of Kalanchoe crenata Andr. Indian J Pharmacol, 2006; 38: 115-119.
7. Divakar MC, Jayaprakasham R. Antiinflammatory and free radical activities of sea cucumber and cuttle fish glandular extracts. Indian drugs, 2006; 43: 471- 475.
8. Kulkarni SK. Hand book of Experimental Pharmacology. Vallabprakasan, 1999; 3: 117-171.
9. Mongeli E, Desmarchelier C, Coussio J, et al. Biological studies of Bolax gummifera, plant of falkland islands used as treatment of wounds. J Ethnopharmacol, 1997; 56: 117-121.
10. Otero R, Nunez V, Barona J, et al. Snakebites and ethnobotany in northwest region of Colombia Part III: neutralization of haemorrhagic effect of Bothrops atrox venom. J Ethnopharmacol, 2000; 73: 233–241.
11. Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S, Kumar AR. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evid. Based Complement. Altern, 2011. Med. DOI: 10.1155/2011/515647
12. Quanico JP, Amor EC, Perez GG. Analgesic and hypoglycaemic activities of *Bixa orellana*, Kyllinga monocephala and Luffa acutangula. Phillip. J. Sci, 2008; 137: 69–76.
13. Radhika B, Begum N, Srisailam K, Feddy VM. Diuretic activity of *Bixa orellana* Linn. Leaf extracts. Indian J. Nat. Prod. Res, 2010; 1: 353–355.
14. Raga DD, Espiritu RA, Shen CC, Ragasa CY. bioactive sesquiterpene from *Bixa orellana*. J Nat Med, 2011; 65: 206–211.
15. Shilpi A, Taufiq-Ur-Rahman M, Uddin SJ, et al. Preliminary pharmacological screening of *Bixa orellana*. J Ethnopharmacol, 2006; 108: 264-271.
16. Shilpi JA, Taufiq-Ur-Rahman M, Uddin SJ, Alam S, Sadhu SK, Seidel V. Preliminary pharmacological screening of *Bixa orellana* L. leaves. J Ethnopharmacol, 2006; 108: 264–271.
17. Silva RB, Almeida CR, Chavasco JM, Chavasco JK. Antimycobacterial activity evaluation and MIC determination of lyophilized hydroalcoholic extracts of *Bixa orellana*

L., Bixaceae. Rev. Bras. Farmacog, 2010; 20. DOI: 10.1590/S0102-695X2010000200006.

18. Tamil Selvi A, DineshMG, Satyan RS, Chandrasekaran B, Rose C. Leaf and seed extracts of *Bixa orellana* L. exert anti-microbial activity against bacterial pathogens. J. Appl. Pharmaceut. Sci, 2011; 1: 116–120.
19. Turner RA, Hebborn P. Screening methods in Pharmacology Academic press, 1971; 2: 210-245.
20. Verdine GL. combinatorial chemistry of nature, Nature, 1996; 384: 11-13.
21. OECD (Organization for Economic Co-operation and Development), 1987. OECD guidelines for testing chemicals, Guideline 423: Acute Oral Toxicity, adopted December 2001; 17: 1-14.