EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 7
615-619
Year: 2020

DETERMINATION OF TOTAL FLAVONOID CONTENT, TOTAL TANNIN, TOTAL PHENOLIC CONTENT AND EVALUATION OF IN VITRO ANTIFUNGAL AND SCREENING AGAINST ASTHMA

Dr. Sweta S. Koka*, Urvashi Lakhani and Dr. G. N. Darhwekar

Acropolis Institute of Pharmaceutical Education and Research Mangliya Indore.

***Corresponding Author: Dr. Sweta S. Koka**
Acropolis Institute of Pharmaceutical Education and Research Mangliya Indore.

Article Received on 19/05/2020 **Article Revised on 09/06/2020** **Article Accepted on 29/06/2020**

ABSTRACT
In this study Hydroalcoholic extracts of *Bixa Orellena* was tested against *Candida Albicans* strain of Fungal. Seeds of *Bixa orellena* was collected from herbal garden of Acropolis Institute of Pharmaceutical Education and Research Indore and were identified according to their macroscopic and microscopic characters. Hydro alcoholic extract of seeds was prepared using Soxhlet method which was further subjected for quantitative estimation of flavonoids, tannins and total phenolics then its In-vitro antifungal and mast cell stabilizing activity was evaluated using Potato Dextrose medium and *In Vitro* Activity Screening Against Asthma By Goat Tracheal Chain Model.

KEYWORDS: *Bixa Orellena* was tested against *Candida Albicans* strain of Fungal.

INTRODUCTION
Asthma is a chronic inflammatory disorder of the airways that causes recurrent episodes of wheezing (sound produced by patient due to suffocation) breathlessness, chest tightness. These symptom are associated with bronchoconstriction and air flow limitation. The cells which are responsible in the inflammatory response are eosinophil, mast cell, and T lymphocyte.[1] The hypertropy of bronchial muscle occurs over a time. The rapid heart rate (tachycardia) and rhonchous lungs sounds, audible through a stethoscope occurs. The cells which are responsible in the inflammatory response are eosinophil, mast cell, and T lymphocyte. The hypertropy of bronchial muscle occurs over a time. The rapid heart rate (tachycardia) and rhonchous lungs sounds, audible through a stethoscope occurs. During severe attacks, the asthamatic sufferer turns blue due to lack of oxygen and become unconscious. The sufferer feet become cold.[2,3]

Mast cells are known to be the primary responders in allergic reactions, most of which are triggered by cross-linking of a high-affinity IgE receptor (FC RI).Allergic manifestations include allergic rhinitis, anaphylaxis, purities and asthma - the diseases associated with inflammatory conditions. Murine systemic anaphylaxis reactions are important parameters for evaluating anti-allergic property. The mast cells have a crucial role in the development of many physiological changes during anaphylactic and allergic responses. Immunoglobulin- E antibodies bind to receptors on the surface of mast cell. Allergen-IgE interaction on mast cell leads to the release of histamine, heparin, proteases and other mediators and the synthesis and secretion of leukotrienes and prostaglandins. These products result in bronchoconstriction, changes in blood vessel tone, increased vascular permeability and myriad other proinflammatory effects. The functions of mast cells can be manipulated for therapeutic ends by regulating their function with appropriate drugs.[4,5]

Candidiasis is most commonly encountered Opportunistic mycosis worldwide. *Candida albicans* is the most common species in the genus which has been implicated in *Candidiasis.* The infections range from superficial of the skin to systemic diseases. *C.albicans, C. tropicalis, C. glabrata and C. parapsilosis* are part of the normal flora of human and can be isolated from oral cavity, vaginal and other parts of body sites from normal healthy peopleUnder certain circumstances, these organisms may gain access to many organ systems such as lung, spleen, kidney, liver, heart, brain, eye, skin and others. Lesions may occur in patients who have disseminated infections.[6,7,8]

Bixa Orellena[9,10,11,12,13]
Bixa orellana L. commonly known as sinduri belongs to the family Bixaceae. It was named after the Spanish conquistador Francisco de Orellana and has been used earlier for body painting, treatment for heartburn and stomach distress, sunscreen, and repelling insects, and to ward off evil. *Bixa orellana* is a tree or shrub to the

tropics of North and South America, the Caribbean, and the East Indies. It is most abundant from Mexico to Ecuador, as well as Brazil, Bolivia, Venezuela, and several other South American countries. It is cultivated in South America and also in south-eastern Asia, where it was introduced by Spaniards in the 17th century. Annatto is a pigment produced by *Bixa orellana* and has been used for centuries in many parts of the world for the prevention and treatment of a number of heath disorders such as constipation, fevers, heartburn, asthma, scabies, ulcers, diarrhoea, stomach upset, skin diseases, measles, anecdotal treatment of diabetes, allergy, leprosy, infectious diseases, burns, measles, gonorrhoea, diarrhoea, asthma, angina, tumours, skin problems, and urinary infections.

Vernacular Names

S.NO	LANGUAGE	NAME
1	SANSKRIT	Sinduri
2	ENGLISH	Lipstick plant
3	BRAZIL	Annato
4	HINDI	Latkan
5	KANNADA	Rangamali
6	TAMIL	Sappiraviraj
7	ORIYA	Lotkon
8	MARATHI	Shendri
9	GUJRATI	Sinduri

Phytoconstituents

- Annatto seeds are covered with aril and contain bixin dye. Annatto seeds are found to contain about 12 per cent of annatto oleo resin of which 50 per cent is water soluble. Volatile oil covers only 0.3-0.8 per cent, while pigment covers 4-5 per cent.
- The main constituent of the pigment is known as bixin which constitutes about 70-80 per cent of pigment. Bixin is a lineal apocarotenoid (Apocarotenoids are terpenoid compounds derived from the oxidative cleavage of carotenoids)of 25 carbon atoms with 9 double bonds and a molecular weight of 394.5 g/mole its molecular empiric formula is C25H30O4, and its scientific name is methyl hydrogen 9′-*cis*-6,6′-diapocaroteno-6,6′-dioate ester.. Seed extracts contain a wide variety of apocarotenoids, including both linear (i.e., methyl (9 Z)-apo-8′-lycopenoate) and cyclic molecules (all-E)-8′-apo-β-caroten-8′-oate).
- Bixin has two different stereochemical configurations: *cis*-bixin and *trans*-bixin. The former *cis* is soluble in most polar organic solvents to which it imparts an orange color and is largely insoluble in vegetable. It may be readily converted to the all-*trans*-isomers due to the instability of the isolated form in solution. *Trans*-bixin is a more stable isomer, it exhibits a red color in solution and is soluble in vegetable oil.

MATERIAL AND METHODS

Collection of Plant Material

Collection of Plant material: Seeds of *Bixa orellena* were collected from Medicinal garden of Acropolis Institute of Pharmaceutical Education and Research, Indore (M.P) and identified the pharmacognosy department on the basis of this morphological and microscopic characters. A voucher specimen has been kept in the herbarium of our department for future references.

Preparation of Extract

250 gm of coarsely powdered seeds were subjected to soxhlet extractor using hydroalcohol (70: 30) as the solvent. The extract thus obtained was filtered, concentrated on water bath, dried in vacuum and stored in refrigerator for further experiment.[14]

Quantitative Phytochemical Screening

Since by the previous literature review it was found the bixa orellena extract contain alkaloid, flavonoid, tannins, glycosides, phenols etc. Thus we had proceeded for Quantitative estimation of Phenols, Flavonoid, Tannin.[15,16,17]

Estimation of Total Phenolic Contents

Total phenolic content was estimated by the following method. 20 µL of the extract was made up to 1 mL with distilled water. 0.5 mL of freshly prepared Folin ciocalteu, phenyl reagent and 2.5 mL of 20% sodium carbonate was added respectively to the extract. The contents were agitated and left in dark for 40 minutes. The absorbance of the sample was read at 725 nm. Gallic acid was used as the standard. Amount of phenolic content was expressed as mg of Gallic acid per gram of plant tissue (mg GA/g).

Estimation of Tannin content

100 µL of extract was made upto 7 mL with distilled water. 8 mM potassium ferric cyanide and 20 mM ferric chloride prepared in 0.1M hydrochloric acid were added respectively. The contents were mixed and optical density was measured at 700 nm. Tannic acid was used as standard. Tannin content was expressed as mg of tannin per gram (mg TA/g).

Estimation of Total Flavonoid Content

Total flavonoid content was estimated by the method of where Quercetin was used as standard. 0.1 mL of extract was taken and made up to 5 mL with distilled water. 0.3 mL of 5% $NaNO_2$ was added. 3 ml of 10% $AlCl_3$ was added after 5 minutes and were shaken well. 2 mL of 1M NaOH was added after 6 minutes and the absorbance was read at 510 nm. The result was expressed as mg quercetin /g.

Antifungal Activity[18,19]

The fungal strain employed in the study was obtained from the Choithram Hospital Pathology, Indore.

Preparation Potato Dextrose Agar Medium

40.0 g of peeled potatoes were cut into small pieces and suspended in 200.0 ml of distilled water which was steamed for 30 min. Decanted the extract through muslin cloth and made the final volume to 200.0 ml to it then 4.0 g of dextrose, 0.02 g of yeast extract and 4.0 g of agar was added to prepare the culture media. Minimum inhibition concentration of each extract using well diffusion method The respective medium was sterilized by autoclaving at 1210C (15lb/ln2) for 15 min and then the medium was transferred aseptically into sterilized glass Petri plates. The plates were left at room temperature to allow for solidification.

Inocolution of Strain

15µl of inoculums of fungi Candida albican was transferred to respective Petri plate. Three wells of 6mm diameter were made using a sterile borer. The different concentrations of drug samples were added with a sterile micropipette to each of the cups. The plates were maintained on sight place for 2 hours to allow the diffusion of the solution into the medium. The Petri dishes are kept inverted position in incubator at 280^{C} for 48 hours. The diameter of zone of inhibition surrounding each of the wells was recorded.

Table: 1 Composition of Potato Dextrose Agar (PDA).

Ingredients	Quantity
Potato infusion	200gm
Dextrose	20gm
Agar	20gm
Distilled water	1000ml

Note: 200 gm of potato infusion is equivalent to 4.0 gm of potato extract.

Determination of Zone of Inhibition

The cultures were grown in nutrient broth and incubated at 37ºc, for 24 hrs. After incubation periods was over, 0.1 ml of culture was seeded in 25 ml molten nutrient agar butts, mixed and poured into sterile petri plates and allowed to solidify. The well was bored with 6 mm borer in seeded agar. 0.1 ml of each extract was added in each well. Plates were kept at 10ºc as a period of pre diffusion for 30 minutes. After it normalized to room temperature; the plates were incubated at 27ºc for 48 hrs for fungi. After incubation period was over, The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well.

***In vitro* activity screening against asthma by goat tracheal chain model.[20]**

Isolated adult goat trachea was collected immediately after slaughter of the animal, then trachea was cut into small individual rings and tied together in series to form a chain. Trachea was suspended in bath of Krebs solution and was continuously aerator at 37^{0}C. Dose Response Curve of histamine in plane Krebs solution and 80ug/ml of plant extract in Krebs solution was taken. Graph of percentage of maximum contractile response on ordinate and concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and in presence of drug extract.

Table 2: Quantitative estimation of phenolic, flavonoid and tannin content.

S.No.	Phytochemical	Concentration Present
1	Total Tannin content	0.317mg/g GAE.
2	Total Phenolic content	0.109mg/g GAE
3	Total Flavonoid content	7.16%.

Table 3 In-Vitro Anti-Fungal Activity Hydroalcoholic Extract of Bixa Orellena

S.no.	Concentration(mg/ml)	Real Concentration(µg)	Zone of diameter (mm) *
1	1	15	3.33 ± 0.577
2	2	30	15.66 ±0.577
3	3	45	16.66 ± 0.577
4	4	60	18.66 ± 1.154
5	5	75	20.33 ± 0.577
6	6	90	12.66 ± 0.577
7	7	105	21.33 ± 0.577
8	8	120	24.33 ± 0.577
9	9	135	20.33 ± 0.577
10	10	150	21.66 ± 0.577

All values represented as Mean ± S.D. (n=3).

Table 4: *In Vitro* Activity Screening Against Asthma by Goat Tracheal Chain Model.

Group	Dose of Histamine (50ug/ml)	Control group Contraction in mm	Test group Contraction in mm	
			Hydroalcoholic extract of Bixa Orellena 200 mg/kg	Hydroalcoholic extract of Bixa Orellena 400 ml/kg
1	0.1	2.753±0.013	1.014±0.120*	1.112±0.334*
2	0.2	3.340±0.167	1.421±0.032*	1.487±0.016*
3	0.4	4.099 ±0.111	1.771±0.115*	1.982±0.186*
4	0.8	6.073 ±0.013	2.344±0.032*	2.265±0.120*
5	1.6	7.466 ±0.117	3.312±0.334*	3.312±0.076*
6	3.2	9.501 ± 0.111	4.111±0.667*	4.212±0.120*

Data are expressed in mean ± S.E.M.
*: p< 0.05 as compared to control

RESULT AND DISCUSSION

Preparation of extract

The hydroalcoholic extract of Bixa Orellana was prepared using Soxhelt method.

Quantitative estimation of Phytochemicals (Phenols, Tannin and Flavonoid)

The total phenolic content was estimated using Gallic acid as standard which is expressed as Gallic Acid equivalent (GAE)/g of tissue where as total tannin content was estimated using tannic acid standard which is expressed as tannic acid equivalent (mg tannic acid (TAE)/ g tissue) and total flavonoid content of the extract was found using quercetin as standard. The result fro the quantitative estimation is shown in Table no. 2.

In-vitro anti-fungal activity

In-vitro anti-fungal activity of Bixa Orellana was determined and zone of inhibition was compared with positive controls. The result obtained is shown in Table no. 3.

In Vitro Activity Screening Against Asthma by Goat Tracheal Chain Model

In goat trachea chain mode extract when administered inhibited the contraction produced by histamine in tissue preparation. Histamine (50ug/ml) was taken in different dose level and DRC was plotted. Study revealed hydroalcoholic extract inhibit significant percentage decreased contraction in goat tracheal chain preparation is shown in table no. 4.

REFERENCES

1. Ian M Adcock, Mathew G John, had found new drugs effective in asthma. Anti IgE therapy was effective in asthma, 1998.
2. Harrison., The Principle of Internal Medicine. Mc Grawhill Publishers, 16 Edition, 2006; 1508-1516.
3. Brian S. A., How Allergies and Asthma Work in the Body. Asthma magazine, 2005; 25-27.
4. Winslow, L. C., Kroll, D. J., Herbs as medicines. Archives of Internal Medicine, 1998; 158: 2192-2199.
5. De, S.P., The role of plant derived drugs and herbal medicines in health care. Drugs, 1997; 54: 801-840.
6. Vealaven S., Nagulendran K. "Invitro antioxidant activity of Asparagus racemosus root", Pharmacog. Mag, 2007; 3: 26-32.
7. Saniasiaya J et al; "Antifunagal Effect of Malaysian Aloe Vera Leaf Extract on Selected Fungal Speciesof Pathogenic Otomycosis Species in Vitro Culture Medium"; Oman Medical Journals, 2017; 32(1): 41-46
8. Shrieen F et al; "Antifungal Activity of Aloe Vera"; Department of Public Health Dentistry, SRM Dental College, Ramapuram, Chennai, Tamil Nadu, India; SRM Journal of Research in Dental Sciences, June 2015; 6: 2.
9. Vilar DA, Vilar MSA, Accioly de Lima e Moura TF, Raffin FN, Rosa de Oliveira M, Franco CLO, et al. Traditional uses chemical constituents and biological activities of Bixa Orellana L. a review. Sci World J, 2014. http://dx.doi.org/10.1155/2014/.
10. Chandel U, Begum T, Syedy M. Pharmacological studies of annatto (Bixa orellana L.). Int J Phar Biomedi Res., 2014; 1(1): 1720.
11. Monzote L, Garcı´a M, Scull R, Cuellar A, Setzer WN. Antileishmanial activity of the essential oil from Bixa orellana. Phytother Res., 2014; 28(5): 753–8.
12. Radhika B, Begum N, Srisailam K, Reddy VM. Diuretic activity of Bixa orellanna Linn. leaf extracts. Ind. J Nat Prod Res., 2010; 1(3): 353–5.
13. Shahid-ul-Islam, Luqman J. Rather, Faqeer Mohammad, Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications – A review, Journal of Advanced Research, 2016; 7; 499–514.
14. R. Mallmanna , E. M. Ethurb , P. Bianchettia , D. Faleiroa , L. Hoehneb , M. I. Goetterta Effectiveness of aqueous and hydroalcoholic extracts of *Acanthospermum australe* (Loefl.) Kuntze against diarrhea-inducing bacteria. Braz. J. Biol., 2018; 78(4): 619-624.
15. Nazish Siddiqui, Abdur Rauf, Abdul Latif, and Zeenat Mahmood, M.D. (Unani) Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e- Zoofa (Nepeta bracteata Benth) Journal of

Taibah University Medical Sciences, 2017; 12(4): 360-363.
16. M. Atanassova, V. Christova-Bagdassarian Determination Of Tannins Content By Titrimetric Method for Comparison Of Different Plant Species Journal of the University of Chemical Technology and Metallurgy, 2009; 44(4): 413-415.
17. Sharma I., Mathur M., Singh G.P., Rishi Anirudha Quantitative estimation of phenolic and flavonoid content and antioxidant activity of various extracts of different parts of Plumbago zeylanica LinnInt. J. Drug Dev. & Res., April - June 2014; 6(2): 136-140.
18. Kumari K, Gupta S. Antifungal properties of leaf extract of Catharanthus roseus l (g.) Don, 2013: 1(9): 698-705.
19. Mahboubi M., Feizabad M., Safara M. Antifungal activity of essential oils from Zataria multiflora, Rosmarinus officinalis, Lavandula stoechas, Artemisia sieberi Besser and Pelargonium graveolens against clinical isolates of Candida albicans. Phcog. Mag, 2008; 4: 15-18.
20. Klaus, J., Erb Domnic, H., Martyres, Peter Seither, Antiasthmatic Agents. Ullmann's Encyclopedia of Industrial Chemistry, 2010.
21. Patil S. D. and Ninave P.B In-Vivo And In-Vitro Screening Models Of Asthma: An Overview. Int. J. Res. Dev. Pharm. L. Sci, June - July, 2016; 5(4): 2209-2218.