WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 1178-1191 Research Article ISSN 2278 – 4357

"INVESTIGATION OF ACTIVE PHYTOCONSTITUENTS OF LEAVES BIXA ORELLANA EXTRACTS"

Rajmani Mafidar*, Dhananjay, Harish Parmar, Rahul Mathur and Dr. Jagdish Rathi

Nri Institute of Pharmaceutical Sciences Bhopal 462021.

Article Received on
12 Feb. 2019,

Revised on 04 Mar. 2019,
Accepted on 25 Mar. 2019

DOI: 10.20959/wjpps20194-13479

***Corresponding Author.**
Rajmani Mafidar
Nri Institute of Pharmaceutical Sciences
Bhopal 462021.

ABSTRACT

The plant *Bixa orellana* Linn., its affined to family Bixaceae. Is a small evergreen shrub or bushy tree commonly known "annatto" in English and "Sinduri" in Sanskrit[1], The prickly heart shaped pods contain small reddish-orange seeds. each pod contains approximately 50 seeds. That is native to central, south and north America. This is concluded that methanolic extract and aqueous extract are more prone to positive result rather than oil extract. So that Bixa orellena will show their positive activity on aqueous and alcoholic extract and many constituet found positive. The crude extract of Bixa orellana leaves was subjected to a preliminary phytochemical screening for the presence of steroids, flavonoids, tannins, saponins, alkaloids, glycoside, cumerin, and carbohydrates. Carcinogenesis is a multistep process, and oxidative damage is linked to formation of tumors through several mechanisms. Oxidative stresses induced by free radicals and cause DNA damage, which, when left unrepaired, can lead to base mutation, single and double strand unseat, DNA cross-linking, and chromosomal damage and rearrangement.

KEYWORDS: Bixa orellana, tannins, sindur, methanol.

INTRODUCTION

The plant *Bixa orellana* Linn., its affined to family Bixaceae. Is a small evergreen shrub or bushy tree commonly known "annatto" in English and "Sinduri" in Sanskrit[1], The prickly heart shaped pods contain small reddish-orange seeds. each pod contains approximately 50 seeds. That is native to central, south and north America. It is also known as achiote or annatto. and it is found mainly in the forest ecosystem of the Brazilian humid tropics but it is now cultivated in the tropics throughout the world.[3] In addition, it enjoys folkloric use in

india, Bangladesh, china and the Philippines. The bixa orellana red seeds serve as the source of a widely used. Red to yellow dye which is referred to as annatto and contains primarily the carotenoids bixin and norbixin. Red dye is used to color and clothes, and various food products including Rice, butter, cheeses, soups, and soft drinks. and make Red body paint, and lipstick, as well as a spice.[5] For this reason, the achiote is sometimes called the lipstick tree. It is mmany medicinal uses in traditional system of medicine including Ayurveda. Root and leaves are useful in the treatment of sore throat, jaundice, liver diseases, diabetes and hypertension.

(***PLANT PROFILE)***

(Bixa orellana)

Kingdom : Plantae

Clade : Angiosperms

Clade : Rosids

Order : Malvales

Family : Bixaceae

Species : B. orellana

Binomial name : Bixa orellana L.

SYNONYMS

- Bixa tinctaria Salisb.
- Orellana orellana (L.) Kuntze
- Bixa americana Poir.

Plant of BIXA ORELLANA

MATERIALS AND METHODS

1. Chemical and reagents

Methanol was obtained from "AVANTOR PERFORMANCE MATERIAL MAHARASHTRA INDIA LIMITED" and distilled water was obtaind from "NRI INSTITUTE LABORATORY BHOPAL" and vegitable oil (soyabin oil) was obtaind from.

2. Plant material and collection

The leaves of Bixa orellana were collected from the plant grown in the botanical garden of department of "NRI INSTITUTE OF PHARMACEUTICAL SCIENCES BHOPAL" after identification by a guide. A voucher specimen is deposited in the laboratory herbarium. The collected plant material was thoroughly checked and free from foreign matter and shade dried. Powdered with laboratory mixer and sieved.

3. Mixing

After shade dried leaf are powder through help of mixure in laboratory.

4. Sieving

After mixing leaf powder are sieving from #22 no sieves. And after sieving make sure powder does not contain any row material. if should be contain. than clean the sample.

5. Prepration of Extract

(a). soxhlet method

The weighed (50g) leaf powder was kept to successive Soxhlet extraction using methanol and water (60-80°C), the solvent should be taken 3/4th of the round bottomed flask, each for 6 hours and each solvent should be extracted single time. The obtained solvent extracts were concentrated and dried in desiccators.

(b). decoction mathod

The weight acuratly (10g) leaf powder was keep for decoction extraction by using vegitable oil (soyabin oil) at room temprature. The solvent should be taken 3/4th of the beackar. for 72 hours. And solvent should be extract after 72 hours. The obtained solvent extract and perform phytochemical test.

PRELIMINARY PHYTOCHEMICAL SCREENING

The crude extract of Bixa orellana leaves was subjected to a preliminary phytochemical screening for the presence of steroids, flavonoids, tannins, saponins, alkaloids, glycoside, cumerin, and carbohydrates.

1. TESTS FOR CARBOHYDRATES

1. Molisch's Test (general test):- To 2-3 ml aqueous extract, added few drops of alpha-naphthol solution in alcohol, shake and add conc. H2SO4 from side of the test tube. Violet ring is fromed at the junction of two liquid.

(A). TESTS FOR REDUCING SUGARS

(a). Fehling's Test:- Mix 1 ml of "Fehling's A" and "Fehling's B" solution boil for one minute. Add equal volume of test solution. Heat in boiling water bath for 5-10 minute. First a yellow, than brick red precipitate is observed.

(b). Benedict's Test:- Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min. solution appears green, yellow or Red depending of amount of reducing sugar present in test solution.

(B). TEST FOR MONOSACCHARIDE:-

(a) Barfoed's test:- Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. in boiling water bath. And cool Red precipitation is observed.

(C). TEST FOR PENTOSE SUGARS:-

(a). Bial's test:- To boiling Bial's reagent add few drops of test solution.green or purpul coloration appears.

(D). TESTS FOR HEXOSE SUGARS

(a). Seliwanoff's test (for keto hexose like fructose):- Heat 3 ml Seliwanoff's reagent and 1 ml.

Test solution in heating water bath for 1-2 minute Red color is formed.

(b). Tollens' Test (phloroglucinol test for galactose):- Mix 2.5 ml conc HCL and 4 ml 0.5% phloroglucinol add 1-2 ml. test solution. Heat yellow to Red color appears.

2. TESTS FOR STEROIDS

(a). Salowski reaction:- To 2 ml of extract, add 2 ml. of chloroform, and 2 ml. conc. H2SO4 shake well, chloroform layer appears Red and acid layer shows greenish yellow fluorescence.

(b) Libermann – Burchard reaction:- Mix 2 ml. extract with chloroform add 1-2 ml. aceteic – anhydride and 2 drops conc. H2SO4 from the side of test tube. First Red, than blue and finally green color appears.

(c). Liebermann's reaction:- Mix 3 ml. extract with 3 ml of aceteic anhydride. Heat and Cool. And few drops of conc. H2SO4. Blue color appears.

3. TESTS FOR ALKALOIDS

Evaporate the aqueous, alcoholic and chloroform extracts separately, To residue, add dilute HCL shake well and filter with filtrate perform following test.

(a). Dragendorff's test:- To 2-3 ml of filtrate, add few drops Dragendorff's reagent. Orange brown precipitate formed.

(b). hager's test:- 2-3 ml.of filtrate with hager's reagent gives yellow precipitate.

4. TESTS FOR GLYCOSIDES

Determine free sugar containt of extract, hydrolise the extract with minerlal acid (dilute HCL/ dilute H2SO4). Again determine the total sugar containt of the hydrolised extract. Increase the sugar containt indicates presence of glycoside in the extract.

(A). TESTS FOR CARDIAC GLYCOSIDES

(a). Baljet test:- Take a piece of lamina or thick section of the leaf and add sodium picrate reagent. If glycoside is present yellow to orange colour will be seen.

(b). Legal test:- To aqueous or alcoholic extract, add 1 ml of pyridine and 1 drops of nitroprusside. Pink to Red colour is appears.

(c). Killer killiani test (test for deoxysugars):- To 2 ml. extract added glacial acetic acid. One drop Of 5% FeCl3 and conc. H2SO4. Raddish brown color appears at junction of the two liquid layer and upper layer appears blueish green.

(B). TESTS FOR ANTHRAQUINONE GLYCOSIDES

(a). Borntranger's test:- To 3 ml of extract, add dilute H2SO4. Boil and filter. To cold filterate add equal volume benzene or chloroform. Shake well. Separate the organic solvent add ammonia. Ammoniacal layer turns pink or Red.

(b). Modified Anthraquinones test:- To 5 ml of extract, add 5ml. 5% Fecl3 and 5 ml diluted HCL Heat for 5 minute in boiling water bath. Cool and add benzene or any organic solvent shake well. Separate organic layer add equal dilute ammonia. Ammoniacal layer shows pinkish Red color.

(C). TESTS FOR SAPONIN GLYCOSIDE

(a). Foam test:- shake the extract or dry powder vigorously with water persistent foam observed.

(b). Heamolytic test:- Add drug extract or dry powder to one drop of blood place on glass slide. " Haemolytic zone appears."

(D). TEST FOR FLAVONOIDS

Shinoda test:- (a). To dry powder or extract, add 5 ml. of 95% ethanol, and few drop of conc. HCL and 0.5g magnesium turnings, pink color observed.

(b). To small quantity of residue, add lead acetate solution yellow colored precipitate is formed.

(c). Addition of increasing amount of sodium hydroxide to the residue shows yellow coloration, which decoloration after addition af acid.

5. TESTS FOR TANNINS AND PHENOLIC COMPOUND

To 2-3 ml of aqueous or alcoholic extract, add few drop of following reagents.

(a). 5% FeCl3 solution:- deep blue-black color.

(b). Lead acetate solution:- white precipitate

(c). Gelatin solution:- white precipitate.

(d). Bromine water:- decoloration of bromine water.

(e). Acetic acid solution:- Red color solution.

(f). Potassium dichromate:- Red precipitate.

(g). Dilute iodine solution:- Transient Red color.

(h). Dilute potassium permanganate solution:- Decoloration.

RESULT AND DISCUSSION

1. TEST FOR CARBOHYDRATES

(a). Molisch's Test:-

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	+ ve
3.	Oil	- ve

(b). Fehling's test(for reducing sugar)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	+ ve
3.	Oil	- ve

(C). Benedict's test

S. No	Solvent	Inference
1.	Methanol	+ ve
2.	Water	+ ve
3.	Oil	- ve

(d). Barfoed's test(for monosaccharide sugar)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	+ ve
3.	Oil	- ve

(e). Bial's test(for pentose sugar)

S. No	Solvent	Inference
1.	Methanol	+ ve
2.	Water	- ve
3.	Oil	- ve

(f). Seliwanoff's test(for hexose sugar):-

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	+ ve
3.	Oil	- ve

(g). Tollens' Test

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	+ ve
3.	Oil	- ve

(2). TESTS FOR GLYCOSIDES

(a). Legal test(for cardiac glycoside)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	- ve
3.	Oil	- ve

(b). Killer killiani test (test for deoxysugars)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	- ve
3.	Oil	+ ve

(c). Borntranger's test(for anthraquinone glycoside)

S. No	Solvent	Inference
1.	Methanol	+ Ve
2.	Water	- Ve
3.	Oil	- Ve

(d). Modified Borntranger's test

S. No	Solvent	Inference
1.	Methanol	+ ve
2.	Water	- ve
3.	Oil	- ve

(e). Foam test(for saponine glycoside)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	- ve
3.	Oil	- ve

(f). Haemolytic test

S. No	Solvent	Inference
1.	Methanol	+ve
2.	Water	- ve
3.	Oil	+ ve

(3). TESTS FOR ALKALOIDS

(a). Dragendorff's test

S. No	Solvent	Inference
1.	Methanol	+ve
2.	Water	- ve
3.	Oil	- ve

(b). Wagner's test

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	- ve
3.	Oil	- ve

(c). Hager's test

S. No	Solvent	Inference
1.	Methanol	+ ve
2.	Water	+ ve
3.	Oil	- ve

(4). TESTS FOR STEROIDS

(a). Salowski test(reaction)

S. No	Solvent	Inference
1.	Methanol	- ve
2.	Water	- ve
3.	Oil	- ve

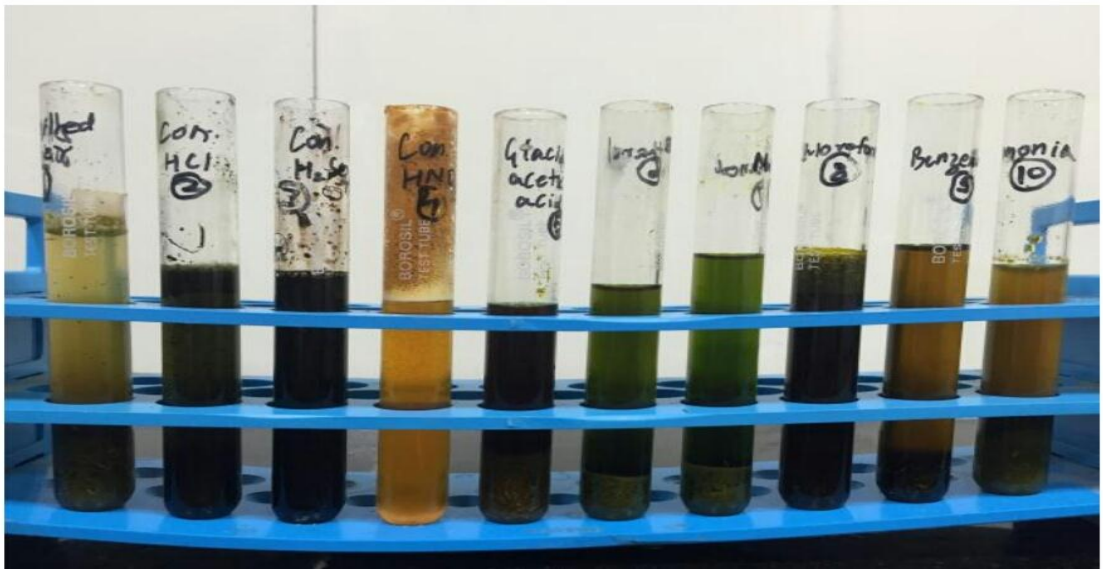
(b). Libermann – Burchard reaction

S. No	Solvent	Inference
1.	Methanol	+ ve
2.	Water	- ve
3.	Oil	- ve

5. TEST FOR FLAVONOIDS

(a). Shinoda test

S. No	Solvent	Step 1	Step 2	Step 3	Step 4
1.	Methanol	- ve	+ ve	- ve	- ve
2.	Water	- ve	+ ve	+ ve	+ ve
3.	Oil	- ve	- ve	+ ve	+ ve



6. TESTS FOR TANNINS AND PHENOLIC COMPOUNDS

S. No	Test (reagents)	Methanol	Water	Oil
1.	**5% FeCl3 solution**	- ve	+ ve	- ve
2.	**Lead acetate solution**	- ve	+ ve	- ve
3.	**Gelatin solution**	+ ve	- ve	- ve
4.	**Bromine water**	ve	Ve	ve
5.	**Acetic acid solution**	- ve	- ve	- ve
6.	**Potassium dichromate**	- ve	- ve	- ve
7.	**Dilute Iodine solution**	- ve	+ ve	- ve
8.	**Dilute HNO3**	- ve	+ ve	- ve
9.	**Dilute potassium permanganate solution**	- ve	- ve	- ve

CONCLUSION

This is concluded that methanolic extract and aqueous extract are more prone to positive result rather than oil extract. So that Bixa orellena will show their positive activity on aqueous and alcoholic extract and many constituet found positive.

Deoxy sugar show the presence in oil extract and hemolytic test is also positive, flavanoid is soluble in oil.

ACKNOWLEDGEMENT

With immense pleasure and profound sense of gratitude I take this golden opportunity to express my heartfelt and sincere intentness to my respected mentor Miss rajmani mafidar and Nri Institute Of Pharmaceutical Sciences Bhopal for giving me best facilities where I carried my research work.

REFERENCES

1. Padhi S., Panda S.K. Phytochemical investigation and antihelmintic activity of various leaf extracts of Bixa orellana Linn., Journal of Innovations in Applied Pharmaceutical Sciences. (JIAPS), 2016; 1(3): 41-45.
2. Deshmukh S.N., Shrivastava B. Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Bixa orenalla linn. Seed. Int. J. Pharm. Sci. Rev. Res., Nov – Dec 2013; 23(1): nº 56, 302-305
3. Tamil S. A., Dinesh M. G., Leaf and Seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens., Journal of Innovations in Applied Pharmaceutical Sciences. (JIAPS), 2011; 01(09): 116-120.

4. Islam S.Ul., Rather L. J., Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications – A review., Journal of Advanced Research, 2015.
5. Mota D. R., Flore L.J. P., chemical characterization and quantification of the pigment extraction yield of seven mexican accessions of bixa orellana caracterizacion qu ´ imica y cuantificaci ´ on del rendimiento de ´ extraccion de pigmento en siete accesiones mexicanas de ´ Bixa orellana., Revista Mexicana de Ingeniería Química, 2016; 15(3): 727-740
6. Kumar A., Lazras A.D., antimicrobial expectation of phytochemical extracted from bixa orellana., World Journal of Pharmaceutical Research, 2016 5(10): 464-467
7. Sangvika S., Malgaonkar M., comparative phytochemical screening of qualitative and quantitative parameters of bixa orellana l., world journal of pharmacy and pharmaceutical sciences, 2015; 4(12): 1001-1017.
8. Radhika B., Begum N., Pharmacognostic and Preliminary Phytochemical Evaluation of the leaves of Bixa orellana., PHCOG J, 2010; 2(7).
9. Ijeoma P., Olaseike O., Phytochemical and Proximate Analysis of Foliage and Seed of Bixa orellana Linn., Int. J. Pharm. Sci. Rev. Res, 2016; 36(2): Article No. 39, 247-251
10. Prathima D., Sujitha A. Phytochemical Screening and Antimicrobial Activity of Bixa Orellana Linn., International Journal of Pharmacognosy and Phytochemical Research, 2016; 8(7): 1078-1082.
11. Kumar Y., babujestadi D., "Preliminary Phytochemical Screening, DNAProtection, Antioxidant and Antiproliferative Effect of Seed Extracts of Bixa orellana L." AJPCT, 2014; 2(8): 1024-1036.
12. Kokate CK, Purohit AP and Gokhale SB, Pharmacognosy, Nirali Prakashan, Edition VII, 1997; 114-116.
13. Kumar S, M. Kabi and M. Kumari, "Study on phytochemical analysis from leaves of Bixa orellana (Kumkum)," Emerging Science, October 2010; 2(5).
14. Patnaik S, Mishra SR and Choudhury GB. Phytochemical investigation and simultaneously study on anticonvulsant, antidiabetic activity of different leafy extracts of Bixa orellana Linn.
15. Jamil Ahmad Shilpi, Taufiq-Ur-Rahman MD, Shaikh Jamal Uddin, Shahanur Alam MD,Samir Kumar Sadhu and Veronique Seidel. Preliminary Pharmacological screening of Bixa orellana L. leaves. J. Ethnopharmacology, 2006; 2(24): 264-271.

16. Bhatnagar, S., S. Swain and M. Sahoo. 2015. Bixa oreallana. L: A study of phytochemical, cytotoxic and antioxidant activity profle of the leaf extracts of the plant. World J. Pharm. Res, 4: 2715-2719.
17. Fleischer TC, Ameade EPK, Mensah MLK and Sawer IK. Antimicrobial activity of the leaves and seeds of Bixa orellana. Fitoterapia, 2003; 74: 136–138.
18. Yong YK, Zakaria ZA, Kadir AA, Somchit MN, Lian GEC, Ahmad Z. Chemical constituents and antihistamine activity of Bixa orellana leaf extract. BMC Complement Altern Med, 2013; 13: 32.
19. Galindo-Cuspinera V, Lubran MB, Rankin SA. Comparison of the volatile compounds in water- and oil-soluble annatto (Bixa orellana L.) extracts. J Agric Food Chem, 2002; 50: 2010–5.
20. Selvi AT, Dinesh MG, Satyan RS, Chandrasekaran B, Rose C. Leaf and Seed extracts of Bixa orellana L. exert anti-microbial activity against bacterial pathogens. J Appl Pharm Sci, 2011; 1 - (09): 116–20.
21. Viuda-martos M, Ciro-go´ mez GL, Ruiz-navajas Y, Zapatamontoya JE, Sendra E, Pe´ rez-a´ lvarez JA, et al. In vitro antioxidant and antibacterial activities of extracts from annatto (Bixa- orellana L.) leaves and seeds. J Food Safety, 2012; 32: 399–406.
22. Shilpi JA, Taufiq-Ur-Rahman M, Uddin SJ, Alam MS, Sadhu SK, Seidel V. Preliminary pharmacological screening of Bixa orellana L. leaves. J Ethnopharmacol, 2006; 108: 264–71.
23. 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.
24. Zuraini A, Somchit MN, Hamid RA, Sukradi S, Fazira AJSE,Yong YK, et al. Inhibitions of acute and chronic inflammations by Bixa orellana leaves extract. Planta Med, 2007; 73: 76.
25. Keong YY, Arifah AK, Sukardi S, Roslida AH, Somchit MN, Zuraini A. Bixa orellana leaves extract inhibits bradykininduced inflammation through suppression of nitric oxide production. Med Princ Pract, 2011; 20: 142–6.
26. Huama´ n O, Sandoval M, Arnao I, Be´ jar E. Antiulcer effect of lyophilized hydroalcoholic extract of Bixa orellana (annatto) leaves in rats. An Fac Med, 2009; 70: 97–102.
27. Fleischer TC, Ameade EPK, Mensah MLK and Sawer IK., "Antimicrobial activity of the leaves and seeds of Bixa orellana", Fitoterapia, 2003; 74: 136-138.

28. Irobi ON, MooYoung M and Anderson WA., "Antimicrobial activity of Annatto (Bixa Orellana) extracts", J Pharmaceutical Biology, 1996; 34(2): 87-90.
29. Deshmukh S N, Shrivastava B, Sharma P, Jain HK, Ganesh N. Pharmacognostical and phytochemical investigation of leaves of Bixa orellana L. Int. J. Pharm. Sci. Rev. Res, 2013; 22(1): 247-252.
30. Yogesh Kumar, Dinesh B, Periyaswamy L. Preliminary phytochemical screening, DNA protection, antioxidant and antiproleferative effect of seed extracts of Bixa orellana L. American Journal of Phytomedicine and Clinical Therapeutics, 2014; 2(8): 1024-1036.
31. Viuda-Martos M, Ciro-Gomez GL, Ruiz-Navajas Y. In vitro antioxidant and antibacterial activities of extracts from annatto (Bixaorellana L.) leaves and seeds,‖ Journal of Food Safety", 2012; 32(4): 399–406.
32. Mohamed Sham, Shihabudeen H, Hansi Priscilla, Kavitha D, Thirumurugan. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research (IJPSR), 2010; 1(10): 430-434.
33. Radhika B, Nasreen BSK. Pharmacognostic and preliminary phytochemical evaluation of the leaves of Bixa orellana, Pharcognosy Journal, 2010; 2(10): 311-316.
34. Stohs SJ, Safety and efficacy of Bixa orellana (achiote, nnatto) leaf extracts, Phytotherapy Research, 2014; 28(7): 956-60. doi: 10.1002/ptr.5088.
35. Biego, G.H.M., Yao, K.D., Koffi, K.M., Ezoua, P. and Kouadio, L.P. Influence of the storage conditions on moisture and bixin levels in the seeds of Bixa orellana L. African Journal of Agricultural Research, 2013; 8: 5342-5347.
36. Sanjeet, K., Kabi, M.,and Kumari, M. Study on phytochemical analysis from leaves of Bixa orellana (Kumkum). Emerging Science, 2010; 2: 1-4.