World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

EFFECT OF SEASONALITY ON ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *ERYTHROXYLUM ARGENTINUM* O. E. SCHULZ AND *ERYTHROXYLUM DECIDUUM* ST. HIL

Arno Ernesto Hofmann Junior[1,2]*, Janaina Tomazin[3], Bruna Maria Saorin Puton[3], Rogério Luís Cansian[3], Jean Carlos Budke[3], Neiva Aparecida Grazziotin[3] and Renata Perreira Limberger[1]*

[1]Toxicology Laboratory, Post Graduate Program in Pharmaceutical Sciences, Universidade Federal do Rio Grande do Sul. Porto Alegre, Rio Grande do Sul, Brasil.
[2]Pharmacognosy Laboratory. Instituto de Desenvolvimento Educacional do Alto Uruguai, Campus II, Getúlio Vargas, Rio Grande do Sul, Brasil.
[3]Biotechnology Laboratory. Universidade Regional Integrada do Alto Uruguai e das Missões. Erechim, Rio Grande do Sul Brasil.

***Corresponding Author: Arno Ernesto Hofmann Junior and Renata Perreira Limberger**
Toxicology Laboratory, Post Graduate Program in Pharmaceutical Sciences, Universidade Federal do Rio Grande do Sul. Porto Alegre, Rio Grande do Sul, Brasil.

Article Received on 22/01/2018 **Article Revised on 12/02/2018** **Article Accepted on 05/03/2018**

ABSTRACT

The genus *Erythroxylum* presents about 240 species in pantropical distribution and the Brazil is the diversity center, with 114 species. The antimicrobial and antioxidant activities were identified for several species of the genus. In the Rio Grande do Sul and Santa Catarina states, South of Brazil, occur mainly *E. argentinum* e *E. deciduum*, in both the presence of some metabolites and the toxicity presented variation with the seasonality. Thus, the influence of climatic stations, summer and winter, about the antimicrobial and antioxidant activities was investigated for different extracts of this species. The study demonstrates wich the activities are influenced by climatic stations, the ethanolic extracts are more actives for the activities investigated and *E. argentinum*, summer collection, presents potential for the discovery of antimicrobial agents against *Staphylococcus aureus*.

KEYWORDS: Seasonality, antimicrobial, antioxidant, *Erythroxylum*.

INTRODUCTION

The genus *Erythroxylum* P. Browne belongs to the family Erythroxylaceae Kunth, present pantropical distribution, comprises about 240 species[1,2,3] and Brazil is the center of diversity and endemism of the genus.[4] In the Rio Grande do Sul and Santa Catarina states, southern Brazil, the most common species of the genus are *Erythroxylum argentinum* O. E. Schulz and *Erythroxylum deciduum* St. Hil. representing about 46% of collections present in Brazilian herbariums and museums. Information obtained through by evaluating the *species*Link system (www.splink.org.br).

The genus present important antimicrobial (*E. coca*,[5] *E. suberosum*,[6] *E. caatinga*,[7] *E. delagoense*, *E. emarginatum*, *E. pictum*,[8] *E. catuaba*[9]) and antioxidant (*E. sideroxyloides*,[10] *E. mummularia*,[11] *E. monogynum*,[12] *E. suberosum* [13]) activities.

E. argentinum present local anesthetic, anti-inflammatory and antimicrobial activities[14,15,16] and *E. deciduum* demonstrated cytotoxicity activity.[17] Both species cause toxicity of sheep when consumed during the summer, a fact that does not occur when consumed in the winter,[18,19,20] chemically, present flavonoids,[15,17,21] alkaloids[22,23,24] and diterpenes.[25] The biosynthesis of metabolites in *Erythroxylum* genus can be influenced by climatic variations and developmental stage.[26,27]

Therefore, the aim of this work was to evaluate the antimicrobial and antioxidant activity of the *E. argentinum* O. E. Schulz and *E. deciduum* A. St. Hil. collected in two different climatic seasons, summer and winter, in Southern of Brazil.

MATERIALS AND METHODS

Collection and Preparation of Extracts

The *E. argentinum* and *E. deciduum* were collected in Brazil, during the summer and winter, in Dense Ombrophylous Forest (27°26'24.0"S 48°22'07.2"W) Santa Catarina state and in transition region between Mixed Ombrophilous Forest and Seasonal Semideciduous Forest (27°39'03.6"S 52°17'56.3"W) Rio Grande do Sul state.

The leaves were separated of the branches, dried, disintegrated and the extracts obtained by exhaustive extraction in a soxhlet apparatus using in increasing polarity the solvents: petroleum ether, dichloromethane, ethanol and ending with the passage of distilled water, the extractions occurred for about one- two hours. The extracts were concentrated and kept in desiccator until constant weight.

Evaluation of Antimicrobial Activity

For the antibacterial activity it were used standard strains of *Enterobacter cloacae* (ATCC 13047), *Enterococcus faecalis* (*Streptococcus faecalis*) (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Proteus mirabilis* (ATCC 25933), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella choleraesuis* (ATCC 10708), *Shigella flexneri* (ATCC 12022), *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228).

The antibacterial activity and Minimal Inhibitory Concentration (MIC) were determined in vitro by agar disc-diffusion method and microdilution in broth.[28,29] To antibacterial activity the microorganisms were standardized with an optical density of McFarland 0.5, corresponds to a concentration of approximately 10^8 UC/mL, which was transferred to Petri dish with Mueller-Hinton agar. Discs of barren paper (6mm) were impregnated with 10 µL of the solvents used in the extraction (negative control) or of the solution 100 mg/mL of the petroleum ether, dichloromethane, ethanol and water extracts from the leaves of *E. argentinum* and *E. deciduum* collected in winter and summer. After placing the discs, the plates were incubated for 18–24 hours at 35ºC. It was named Sensitive when the inhibition halo was equal to or greater than 7 mm and Resistant when no occurred inhibition halo.

The MIC was considered the lowest concentration of extract that no microbial growth occurred. In microplates of 96 wells, each well received 100 µL Mueller-Hinton broth, 100 µL extract and a suspension of 10^4 UFC/well of the microorganism. The negative control occurred employing only broth and extract and to positive control only suspension of microorganism and broth. The experiments were performed in triplicate.

Evaluation of Antioxidant Activity

The methodology performed is based on the extent of the extinction of the radical absorption 2,2-diphenyl-1-picrylhydrazyl (DPPH) in spectrophotometer at 515 nm, the determination occurred in UV-Visible spectrophotometer brand Agilent Technologies, model 8453E. After the evaluation of the ideal concentration range, was calculated the concentration of extracts required to capture 50% of the DPPH free radical (EC50) by linear regression analysis.[30] The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Antimicrobial Activity

The results of the antibacterial activity of different extracts leaves of *E. argentinum* and *E. deciduum*, collected from two different climatic seasons, summer and winter, using the disc diffusion method, are presented in **Table 1**.

Table 1: Antibacterial activity, in disc diffusion method, of the extracts of leaves of *Erythroxylum deciduum* and *Erythroxylum argentinum* collected in winter and summer using 10 µL of a solution at a concentration close to 100 mg/mL.

Microorganisms	Extracts							
	EP		CH2Cl2		EtOH		H2O	
	W	S	W	S	W	S	W	S
Enterobacter cloacae	-	-	-	-	-	-	-	-
Enterococcus faecalis	-	-	-	A(8)	D(7) A(9)	D(9) A(10)	-	-
Escherichia coli	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	-	-	-	-	-	-	-	-
Proteus mirabilis	-	-	-	-	-	D(8)	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-	-
Salmonella choleraesuis	-	-	-	-	A(8)	D(8) A(9)	-	-
Shigella flexneri	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	-	A(8)	A(8)	D(8) A(10)	D(9) A(12)	A(8)	A(8)
Staphylococcus epidermidis	-	-	-	-	A(8)	A(11)	-	-

-: Resistent, A: sensible to E. argentinum. D: sensible to E. deciduum. ( ): Diameter of the halo of inhibition of microbial growth in millimeter, EP: extract petroleum ether, CH2Cl2: extract dichloromethane, EtOH: extract ethanolic, H2O: extract water, W: collect in winter, S: collect in summer.

E. argentinum and *E. deciduum* were presented antibacterial activity against *E. faecalis*, *S. choleraesuis* and *S. aureus*. For *P. mirabilis* only *E. deciduum* was active and for *S. epidermidis* only *E. argentinum*. Similar to our results, *E. caatinga* demonstrates activity against *S. aureus* and *E. faecalis* and shows no activity against *E. coli* and *P. aeruginosa*.[7]

The antibacterial activity, in disc diffusion method, of extracts leaf of *E. deciduum* and *E. argentinum* demonstrate that the seasonality, winter/ summer, interferes about this activity of the species, being more active in the summer.

The ethanolic extracts produces more inhibition haloes against the microorganisms studied as the study in *E. delagoense*, *E. emarginatum* and *E. pictum.*[8]

The apolar extracts present low or no antibacterial activity, characteristic also observed in other species of genus as *E. caatinga* and *E. suberosum.*[6,7]

The active extracts in disc diffusion method (zone of inhibition > 7 mm) were evaluated by broth microdilution method, the values MIC obtained are shown in **Table 2** and **Table 3.**

Table 2: Minimal Inhibitory Concentration (MIC) in mg/mL of extracts dichloromethane, ethanolic and aqueous of leaves of *Erythroxylum argentinum*, collect in winter and summer.

Microorganisms	CIM (mg/mL) – W			CIM (mg/mL) – S		
	CH2Cl2	EtOH	H2O	CH2Cl2	EtOH	H2O
Enterococcus faecalis	-	6.25	-	12.537	3.125	-
Salmonella choleraesuis	-	12.5	-	-	3.125	-
Staphylococcus aureus	6.331	3.125	6.287	6.269	0.78125	3.147
Staphylococcus epidermidis	-	12.5	-	-	3.125	-

CH2Cl2: extract dichloromethane, EtOH: ethanolic extract, H2O: water extract, (W): collect in winter, (S): collect in summer and (-): not evaluated.

Table 3: Minimum Inhibitory Concentration (MIC) in mg/mL of ethanolic extracts from leaves of *Erythroxylum deciduum*, collect during winter and during the summer.

Microorganisms	CIM (mg/mL) - W	CIM (mg/mL) – S
Enterococcus faecalis	6.252	3.138
Proteus mirabilis	-	12.552
Salmonella choleraesuis	-	6.276
Staphylococcus aureus	3.126	3.138

(W): collect in winter and (S): collect in summer and (-): not evaluated.

According to De Wet[8] and Van Vuuren,[31] extracts with MIC value below 8 mg/mL are considered active and below 1mg/mL true antimicrobials. In this way, the species evaluated demonstrate antibacterial activity and potential for the discovery of antimicrobial molecules, being *E. argentinum*, collected in summer, the most promising, MIC= 0.78125 mg/mL against *S. aureus.*

S. aureus was the microorganism most sensitive to extracts, this result is important because the bacterium is the main human pathogen, responsible for serious infections, increasing resistance to antimicrobials and their clinical spectrum of diseases to be mutable,[32] so finding new sources for their treatment is important.

The absence of activity against *E. coli*, *K. pneumoniae* and *P. aeruginosa* in our study with *E. argentinum* corroborate the results of Tomesi[14] for the same species. However occurs divergence on *S. aureus*, which may originate from the collection period or other edaphoclimatic factors or error in identification of the species.

Practically all extracts collected in summer present more antimicrobial activity that the respective extracts collected in winter. These difference demonstrate the seasonal influence in the biosynthesis of compounds responsible for the activity in these plants, being more synthesized/ accumulated in the summer.

The extracts tested were more active on gram positive, the result is in agreement with others studies that demonstrate the gram negative as the more resistant to the action of the vegetal extracts.[6,33,34] This may be related with the presence of lipoproteins in the membrane, which causes deficiency in the penetration and thus inactivity or reduction of activity.[35] Ethanolic extracts were active against the gram negative *S. choleraesuis* and *P. mirabilis*, which suggests the production of metabolites with capacity to transpose the lipoproteins or present an alternative mechanism of action or penetration.

It is observed that almost all studies do not present information about the seasonality and the collection site, which impairs the comparison between studies once antimicrobial activity may vary between summer and winter.

Antioxidant Activity

The results obtained of the antioxidant activity, in the form of EC50, of the different extracts of the *E. argentinum* and *E. deciduum* collected in two climatic seasons, summer and winter, are presented in **Table 4**.

Table 4: Antioxidant activity presented as EC50 of extracts leaves of *Erythroxylum argentinum* and *Erythroxylum deciduum* collected in winter and summer.

EC50 (mg/ml)					
Species	**Collect**	**Extract**			
		EP	**CH2Cl2**	**EtOH**	**H2O**
Erythroxylum argentinum	Summer	0.96	0.05	0.03	0.04
	Winter	0.44	0.10	0.02	0.07
Erythroxylum deciduum	Summer	0.99	1.16	0.04	0.10
	Winter	0.65	0.24	0.04	0.13

EP = petroleum ether extract; CH2Cl2 = dichloromethane extract; EtOH = ethanolic extract and H2O = water extract.

The polar extracts of *E. argentinum* and *E. deciduum* present antioxidant activity and the ethanolic extracts were the most active, similar to the results found in *E. cuneatum.*[35]

The apolar extracts presented low antioxidant activity, similar to others studies with species of the genus,[36] however the dichloromethane extract of *E. argentinum*, collected in summer, had na interesting antioxidant activity.

The seasonality does not present important influence on the antioxidant activity of ethanolic extracts of *E. argentinum* and *E. deciduum*, but about the activity of the water extracts, this influence occurs.

The antioxidant activity of *E. monogynum*, EC50= 0.05 mg/mL, is responsible for protection of the plant against hepatic damage,[12,37] therefore *E. argentinum*, EC50= 0.02 mg/mL, and *E. deciduum*, EC50= 0.04 mg/mL, present potential for therapeutic use. Due antioxidants compounds present several therapeutic properties such as anti-inflammatory, anticancer and vasodilator,[10] the species, *E. argentinum* and *E. deciduum*, also demonstrate are possible source of the news pharmaceutical molecules in the market.

CONCLUSION

Erythroxylum argentinum and *Erythroxylum deciduum* present antioxidant and antimicrobial activity, especially against gram positive bacteria and *Erythroxylum argentinum* present potential for clinical use against *S. aureus.*

The antimicrobial and antioxidant activities of the species evaluated present variation with the seasonality, this demonstrate the need for inform this period of collect in publications, since an interesting activity may not be verified due the influence of the seasonality.

FUNDING

The authors thanks the financial support of CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico and CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazil).

REFERENCES

1. Cronquist A. The Evolution and Classification of Flowering Plants. Second Edition. Bronx, NY: The New York Botanical Garden, 1988.
2. Souza VC, Lorenzi H. Botânica Sistemática: guia ilustrado para identificação das famílias de Fanerógamas nativas e exóticas no Brasil, baseado em APG III. 3ª Edição, Nova Odessa, São Paulo: Instituto Plantarum de Estudos da Flora Ltda, 2012.
3. Costa-Lima JL, Loiola MIB, Jardim JG. Erythroxylaceae of Rio Grande do Norte, Brazil. Rodriguésia, 2014; 65: 659-71.
4. Lucas-Filho MD, Silva GC, Cortes SF, Mares-Guia TR, Ferras VP, Serra CP, Braga FC. ACE Inhibition by Astilbin Isolated from *Erythroxylum* gonocladum (Mart.) O.E. Schulz. Phytomedicine, 2010; 17: 383-7.
5. Ventura G, Castro A, Roque M, Ruiz J. Composición Química del Aceite Esencial de *Erythroxylum coca* Lam var. *coca* (coca) y Evaluación de su Actividad Antibacteriana, Ciencia e Investigación, 2009; 12: 24-8.
6. Violante IMP, Hamerski L, Garcez WS, Batista AL, Chang MR, Pott VJ, Garcez FR. Antimicrobial Activity of Some Medicinal Plants from the Cerrado of the Centralwestern Region of Brazil. Braz J Microbiol, 2012; 1302-8.
7. Aguiar JS, Araújo RO, Rodrigues MD, Sena KXR, Batista AM, Guerra MMP, Oliveira SL, Tavares JF, Silva MS, Nascimento SC, Da Silva TG. Antimicrobial, Antiproliferative and Proapoptotic Activities of Extract, Fractions and Isolated Compounds from the Stem of *Erythroxylum caatingae* Plowman. Int J Mol Sci, 2012; 13: 4124-41.
8. De Wet H. Antibacterial activity of the five South African Erythroxylaceae species. Afr J Biotechnol, 2011; 10: 11511-14.
9. Manabe H, Sakagami H, Ishizone H, Kusano H, Fugimaki M, Wada C, Komatsu N, Nakashima H, Murakami T, Yamamoto N. Effects of Catuaba extracts on microbial and HIV infection. In Vivo, 1992; 6: 161-5.
10. Soobrattee MA, Bahorun T, Neergheen VS, Googoolye K, Aruoma OI. Assessment of the Phenolic and Antioxidant Actions of the Rubuaceae, Ebenaceae, Celasatraceae, Erythroxylaceae and

Sterculaceae Families of Mauritian endemic plants. Toxicol In Vitro, 2008; 22: 45-56.
11. Barreiros ALBS, Barreiros ML, David JM, Davis JP, de Queiroz LP. Atividade antioxidante e substâncias presentes em *Dioclea violaceae* e *Erythroxylum mummularia* Rev Bras Farmacogn, 2003; 13(supl 2): 8-11.
12. Syed SH, Namdeo AG. Hepatoprotective effect of leaves of *Erythroxylum monogynum* Roxb. on paracetamol induced toxicity. Asian Pac J Trop Biomed, 2013; 3: 877-81.
13. Barros IMC, Leite BHM, Leite CFM, Fagg CW, Gomes SM, Resck IS, Fonseca-Bazzo YM, Magalhães PO, Silveira D. Chemical composition and antioxidant activity of extracts from *Erythroxylum suberosum* A. St. Hil. Leaves. J Appl Pharm Sci, 2017; 7: 88-94.
14. Tomesi CN, Viale AA, Buschi CA, Roft RD, Schteingart CD, Iñigo RPA, Zallocchi EM, Pomilio AB. Antimicrobial Screening of Some Argentine Higher Plants. Fitoterapia, 1986; LVII: 46-50.
15. Chaves GG, Schapoval EES, Zuanazzi JA, Diehl E, de Siqueira NCS. *Erythroxylum argentinum*: assays for anti-inflammatory activity. J Ethnopharmacol, 1988; 22: 117–20.
16. Zuanazzi JAS, Rates SMK, Henriques AT. Cocaine-like Actions of *Erythoxylum argentinum* Schultz (Erythroxylaceae). Acta Farm Bonaerense, 2000; 19: 105–8.
17. Nascimento GC, Menezes ACS, Lacerda EPP. Identificação do Flavonóide 7,4'-Dimetoxi-Quercentina-3-O-B-D-Glicopiranosídeo e Avaliação da Atividade Antitumoral dos Frutos. Revista Processos Químicos, 2011: 44-55.
18. Barros RR, Teixeira FR, Oliveira FN, Rissi DR, Rech RR, Barros CS. Poisoning in sheep from the ingestion of fruits of *Erythroxylum argentinum*. Vet Hum Toxicol, 2004; 46: 173-5.
19. Borelli V, Lentz D, Veronezi LO, da Silva TGE, Kaufer L, Traverso SD, Gava A. Intoxicação espontânea e experimental por folhas e frutos de *Erythroxylum deciduum* (cocão) em ovinos no Estado de Santa Catarina. Pesq Vet Bras, 2011; 31: 213-8.
20. Colodel EM, Seitz AL, Schmitz M, Borba MR, Raymundo DL, Driemeier D. Intoxicação por *Erythroxylum deciduum* (Erythroxylaceae) em ovinos. PesqVet Bras, 2004; 24: 165-8.
21. Inigo RPA, Pomilo AB. Flavonoids from *Erythroxylum argentinum*. Phytochemistry, 1985; 24: 347-9.
22. Bieri S, Brachet A, Veuthey JL, Christen P. Cocaine distribution in wild *Erythroxylum* species. J Ethnopharmacol, 2006; 103: 439–47.
23. Oliveira SL, da Silva MS, Tavares JF, Sena-Filho JG, Lucena HFS, Romero MAV, Barbosa-Filho JM. Tropane Alkaloids from *Erythroxylum* Genus: Distribution and Compilation of ^{13}C-NMR Spectral Data. Chem Biodivers, 2010; 7: 302-26.
24. Zuanazzi JAS, Tremea V, Limberger RP, Sobral M, Henriques AT. Alkaloids of Erythroxylum (Erythroxylaceae) species from Southern Brazil. Biochem Syst Ecol, 2001; 29: 819–25.
25. Ansell SM, Pegel KH, Taylor DAH. Diterpenes from the Timber of 20 *Erythroxylum* Species. Phytochemistry, 1993; 32: 953-9.
26. Brock A, Bieri S, Christen P, Dräger B. Calystegines in wild and cultivated *Erythroxylum* species. Phytochemistry, 2005; 66: 1231-40.
27. Docimo T, Reichelt M, Schneider B, Kai M, Kunert G, Gershenzon J, D´Auria JC. The first step in the biosynthesis of cocaine in *Erythroxylum coca*: the characterization of arginine and ornithine decarboxylases. Plant Mol Biol, 2012; 78: 599-615.
28. NCCLS (*National Committee for Clinical Laboratory Standard*). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard – Sixth Edition.* NCCLS document M7-A6, Wayne, Pennsylvania, USA, 2003; 23(2).
29. NCCLS (*National Committee for Clinical Laboratory Standard*). *Performance standards for antimicrobial disk susceptibility tests: approved standard-eighth Edition*. NCCLS document M2-A8, Wayne, Pennsylvania, USA, 2003; 23(2).
30. Silvestri JDF, Paroul N, Czyewski E, Lerin L, Rotava I, Cansian RL, Mossi A, Toniazzo G, De Oliveira D, Treichel H. Perfil da composição química e atividades antibacteriana e antioxidante do óleo essencial do cravo-da-índia (*Eugenia caryophyllata* Thunb.). Rev Ceres, 2010; 57: 589-94.
31. Van Vuuren SF. Antimicrobial activity of South African medicinal plants. J Ethnopharmacol, 2008; 119: 462-72.
32. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler JVG. Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbiol Rev, 2015; 28: 603-61.
33. De Albuquerque CK, Tavares JF, De Oliveira SL, Silva TS, Gonçalves GF, Costa VCO, Agra MF, Pessôa HLF, da Silva MS. Flavonoides Glicosilados de *Erythroxylum pulchrum* A. St.-Hil. (Erythroxylaceae). Quim Nova, 2014; 37: 663-6.
34. Bussmann RW, Malca-García G, Glenn A, Sharon D, Chait G, Díaz D, Pourmand K, Jonat B, Somogy S, Guardado G, Aguirre Chan CR, Meyer K, Kuhlman A, Townesmith A, Effio-Carbajal J, Frías-Fernandez F, Benito M. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. J Ethnopharmacol, 2010; 132: 101-8.
35. Kadchumsang S, Sirisa-Ard P, Sookkhee S, Chansakaow S. Antibacterial and Antioxidant Activities of Lanna Medicinal Plants Used in Mahoog Formula. Int J Pharm Pharm Sci, 2015; 7: 366-70.

36. Córdova WHP, Matos MG, Tabart J, Sipel A, Kevers C, Dommes J. In Vitro Characterization of Antioxidant Properties of Cuban Endemic Varieties of *Erythroxylum alaternifolium* A. Rich. Isolation of Two Flavonol Glycosides. J Chil Chem Soc, 2012; 57: 1340-43.
37. Kumar VR, Reddy GV, Krishna MR. Screening of Phytochemicals and Antioxidant Activity of *Erythroxylum monogynum*. Int J Bioassays, 2014; 3: 3005-7.