International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (1): 1315-1321
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Preliminary Phytochemical Screening and *in vitro* Antioxidant Activity of *Eugenia uniflora* L. Leaf Extracts

S. Syama[a], Lal Raisa Helen[a] and MS Latha[a]*
School of Biosciences, M.G. University, Kottayam, Kerala, India-686560

Received: 12 Oct 2018 / Accepted: 18 Nov 2018 / Published online: 1 Jan 2019
Corresponding Author Email: mslathasbs@gmail.com

Abstract
The main aim of this work is to investigate the phytochemicals present in *Eugenia uniflora* and also its free radical scavenging activity of various *Eugenia leaf* extracts. The phytochemical screening was conducted by standard methods and free radical scavenging activity was estimated by DPPH, FRAP, ABTS and Total antioxidant assays. The results of phytochemical screening revealed the presence of secondary metabolites in *Eugenia uniflora* leaf extracts. Alkaloids, tannins, flavonoids, saponins, steroids, terpenoids and glycosides are present in leaf extracts of *Eugenia uniflora*. Results of antioxidant assay shows that all the extracts of *Eugenia uniflora* had free radical scavenging ability but compared to other extracts ethanolic extracts showed increased antioxidant activity. From the above results of phytochemical screening and antioxidant assay it can be concluded that *Eugenia uniflora* contain an enormous amount of phytochemicals. Presence of these phytochemicals are responsible for the free radical scavenging activity of *Eugenia uniflora* L. All these properties contribute *Eugenia uniflora* to act as a good therapeutic drug.

Keywords
Phytochemicals, free radical scavenging, Glycosides, *Eugenia uniflora*, saponins, Flavonoids.

*****

1. INTRODUCTION
Medicinal plants are achieving priorities in both developed and developing countries for the treatment of various human health disorders. Naturally occurring compounds present in plants are called phytochemicals. Plant selected for this work is *Eugenia uniflora* L. *Eugenia uniflora* belongs to the family Myrtaceae and is native to South America .Flowers of *Eugenia* are cream in colour .*Eugenia uniflora* is the only plant in myrtaceae family that bears edible fruit .The colour of the *Eugenia* Fruit ranges from green to dark red.The fruit becomes black in colour as it ripens more.*Eugenia uniflora* is also known as pitanga or Surinam cherry. Young leaves of *Eugenia uniflora* are pinkish in colour as they grow the colour changes to dark green. Leaf of *Eugenia uniflora* is used for making tea. This tea can be used as folk medicine against fever, lowering blood pressure and also for infection. *Eugenia uniflora* has anti-inflammatory, antioxidant, antidiabetic and anticancer property. The part of the plant used for this study was its leaves.
Here in this work *Eugenia* leaves were washed, shade dried and powdered. This leaf powder was extracted

with various solvents such as petroleum ether, chloroform, ethylacetate, ethanol, methanol and water by using soxhlet extraction. Solvents used for extraction were selected on the basis of polarity. After this all the extracts were subjected to qualitative phytochemical screening in order to detect the presence of secondary metabolites. Finally, all the extracts were subjected to different antioxidant assay to detect their free radical scavenging property.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Plant was collected from Alapuzha district(kerala)and was identified. The part of the plant used for this study is leaf.

2.2 Preparation of plant extract

Eugenia uniflora leaf extract was shade dried and then grinded into fine powder. This powder was then extracted using various solvents of increasing polarity. Soxhlet method used for extraction. After extraction extracts of various solvents was dried and then subjected to phytochemical and antioxidant assays.

2.3 Chemicals:

The chemicals used for this work includes DMSO (Dimethyl sulphoxide), Ascorbic acid,28Mm Sodium phosphate,4Mm Ammonium molybdate, DPPH (2,2, Diphenyl dipicryl hydrazine, 300Mm Sodium acetate, 10Mm TPTZ, 40Mm Hydrochloric acid, 20Mm Ferric chloride,and Ferrous sulphate

2.4 Qualitative analysis of phytochemicals [16].

2.4.1 Alkaloids

a. Mayer'sTest:

0.5g of the leaf extract was stirred with few ml of dilute hydrochloric acid and filtered. To a few ml of filtrate, one or 2 drops of mayer's reagent was added along the sides of the test tubes. A white creamy precipitate demonstrated the test as positive.

b.Dragendroff's reagent:

A-Add 0.17g Bismuth Nitrate in 2ml acetic acid and dilute to 10ml with distilled water.

B-4g potassium iodide in 10ml acetic acid and dilute to 20ml with distilled water.

To the plant extracts few drops of Dragendroff's reagent was added. The formation of orange red colour confirms the presence of alkaloid.

2.4.2 Steriods:

1g of leaf extract was dissolved in a few drops of chloroform. It was then gently warmed and then cooled under the tap water. And then add a drop of concentrated sulphuric acid along the sides of the test tube. Appearance of reddish-brown colour at the interface indicates the presence of steroids.

2.4.3 Flavonoids

a.Shinoda test:

Crude leaf extract was mixed with few fragments of magnesium ribbon and concentrated hydrochloric acid was added dropwise. Pink scarlet colour appears after few minutes which indicated the presence of flavonoids.

b. Lead acetate test:

Leaf extract treated with few drops 1%lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.4.4 Terpenoids:

Crude leaf extract was dissolved in 2ml chloroform and then evaporated to dryness. To this 2ml concentrated sulphuric acid was added and heated for 2minutes. Formation of grayish colour indicates the presence of terpenoids.

2.4.5 Glycosides:

a. Salkwoski's test:

Leaf extract was dissolved in 2ml of chloroform sulphuric acid was then carefully added to form a lower layer. A reddish-brown colour at the interface demonstrated the presence of glycosides.

2.4.6 Saponins:

a. Frothing test:

To 1 gram of leaf add 3ml of distilled water and shaken vigorously for about 5 minutes. Frothing which persisted for 10 minutes indicates the presence of saponins.

2.4.7 Tannins:

a. Gelatin test:

To the extract 1%gelatin solution containing 10%sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.5. *IN VITRO* ANTIOXIDANT ASSAYS:

1. DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity):

The method used for DPPH radical scavenging activity was by Kiranmai et al, with some modification. Here 2ml of reaction mixture containing 1ml of DPPH (100uM in methanol) and various concentration of 1ml of leaf extract was incubated for 30 minutes at 37°C. Absorbance was read at 517nm.Percentage inhibition of DPPH radical was calculated by comparing the results of test with control using the equation. [16].

$$\% \text{ of DPPH radical scavenging activity(\%RSA)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where 'A' is the absorbance.

2.5.2 FRAP (Ferric Reducing Antioxidant Power assay)

0.2ml of the test sample was mixed with 1.8ml of frap reagent. This is then incubated at 37^0C for 10 minutes in a water bath .FRAP reagent involves 0.3Macetate buffer with pH 3.6, 20mmTPTZ solution ,and 20mm$Fecl_3.6H_2O$.Absorbance was read at 593nm after the incubation .The above reaction mixture without the leaf extract serves as the control for this assay. Standards used for this assay is Methanolic solution of known Fe (II)concentration. The values of the results were expressed as mmol Fe (II) of extracts. All the assay was carried out in triplicate. Mean values of +/- SEM were also done. [16].

2.5.3 Total Antioxidant activity:

The method used for identifying total antioxidant activity was phosphomolybdenum method. This was developed by prieto and colleagues. (prieto-etal). Principle behind this assay is the reduction of Mo(V_1)-Mo(V) by the given extract that results in the formation of green coloured phosphate /Mo (v) complex. This complex is formed at acidic pH. Here 0.3ml extract was combined with 3ml of 0.06Msulfuric acid ,28mM sodium phosphate and 4Mm ammonium molybdate that act as reagent solution .All the tubes that contain reaction solution was incubated at 20 minutes at 95°C in water bath .After incubation absorbance was read at 695nm against blank .Only after cooling the test tubes to room temperature .Blank used in this assay is 0.3ml methanol. The number of equivalents of ascorbic acid is used to express antioxidant activity.[19].

2.5.4[2, 2'-azinobis (3-ethyl benzothiazolines sulphonic acid)]

ABTS **radical scavenging assay**

Distilled water (1ml) was added to 0.2ml of different concentrations of the samples (125-2000ug/ml).

From a stock concentration of 10mg/ml and 0.16ml of ABTS was added. Final volume was made up to 1.36ml.A control without test was also taken .Control was made up of equal amounts of distilled water. All the tubes were incubated for 20 minutes. After incubation absorbance was taken at 734 nm. [17].

Calculation:

% inhibition = $\frac{control-test}{control} X100$.

2.6 Statistical analysis:

The arithmetic mean was compared using analysis of variance (ANOVA) and Tukey Test using a software programs Graph pad prism 5.

3. DISCUSSION:

Phytochemicals in plants beneficial for human health. Phytochemicals have role in plant defence mechanism. They are also known as phytonutrients. Qualitative phytochemical screening of *Eugenia uniflora* leaf showed the presence of different phytochemicals in various extracts **(Table 1).** Solvent used for extraction have significant role on the phytochemical analysis. From the results of the present study it was vibrant that petroleum ether and chloroform extracts showed the presence of terpenoids, steroids and glycosides. Water extract also showed the presence of glycosides and absence of steroids. Ethyl acetate extract possess high of flavonoid content. In case of ethanolic and methanolic extracts there was an abundance in flavonoids, saponins and tannins. Alkaloid was abundant in ethanolic extract. Alkaloid was present in case of water and methanolic extract. Terpenoids, glycosides and steroids were absent in ethanolic, methanol extracts. Qualitative phytochemical analysis was done to detect the presence of secondary metabolites. All the assays were carried using standard procedures.

Free radicals are responsible for most of the diseases that are affected by humans. Antioxidants have the ability to scavenge these free radicals that cause damage to cell. In order to retain health antioxidants are needed. Antioxidant assay was performed mainly to find whether the *Eugenia uniflora* leaf extract had the ability to scavenge free radicals. Most of the secondary metabolites have antioxidant property. The assays performed include DPPH assay, Frap assay, Total antioxidant assay and ABTS assay.

In the case of DPPH assay the ethanolic extract(E) of *Eugenia uniflora* showed highest value 76.82%. The standard used for this assay was ascorbic acid (ASC). The other extracts of *Eugenia uniflora* showed percentage inhibition lower than that of *Eugenia uniflora* ethanolic extract. Values of other extracts were follows; PE=22.5%, CH=34.2%, EA=42.5%, W=48.5%, M=56.04%, and ASC=86.6%. (Fig 1). All the extracts exhibited DPPH radical scavenging activity. On comparing the activity exhibited by these extracts with standard ascorbic acid it can be concluded that *Eugenia uniflora* ethanolic extract showed highest DPPH scavenging activity and petroleum ether exhibited lowest activity. IC_{50} of all the extracts including the ascorbic acid are shown in the fig 2.

In FRAP assay all the solvent extracts of *Eugenia uniflora* possessed free radical scavenging activity. ethanolic extract exhibited highest ferric reducing power activity than the other solvent extracts of *Eugenia uniflora*. Values obtained by this assay from

petroleum ether solvent extract to ethanolic extract as follows, PE=1.024%, CH=1.35%, EA=2.147%, M=3.465%, W=5.46%, E=7.46%and standard ascorbic acid =10.6%. Highest ferric reducing power activity was displayed by the ethanolic extract(E)and lowest by petroleum ether extract. (fig 3).

Total antioxidant assay was also performed using all these solvent extracts. Outcome of the result indicate that maximum activity was shown by the ethanolic extract than the other solvent extracts of *Eugenia uniflora*, and also the lowermost antioxidant activity was by petroleum ether. The values acquired were PE=9.29%, CH=11.46%, EA=12.39%, M= 13.39%, W= 13.3%, E=17.25% and ascorbic acid=22.46%. (fig 4).

All the *Eugenia uniflora* solvent extracts were then subjected to the ABTS for checking the free radical scavenging activity. In this ABTS assay, also all of the *Eugenia uniflora* extracts showed antioxidant activity. It was remarkable that Ethanolic extract possessed uppermost values compared to others and Petroleum ether with lowermost free radical scavenging activity on comparing with standard ascorbic acid. Values shown by the extracts and standard were PE=6.4%, CH=10.2%, EA=14.5%, M=28.8%, W=32.67%, E=40.07%, ASC=56.01%. (fig 5). On behalf of the data obtained from this study, it is clear that ethanolic extract of *Eugenia uniflora* was found to be effective in scavenging free radicals. The results of the study were very encouraging as the leaf extract showed the presence of phytochemicals and because of these phytochemicals the leaf is having good antioxidant potential.

	Petroleum Ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Methanol extract	Water extract
Alkaloids	–	–	–	+++	+	+
Flavanoids	–	–	+++	+++	+++	+++
Saponins	–	–	–	+++	+++	++
Tannins	–	–	–	+++	++	+++
Terpenoids	++	++	–	–	–	+
Steroids	++	++	–	–	–	–
Glycosides	++	++	–	–	–	++

* Key : - = Absence , + = small presence / Presence , ++ = Plenty/present , +++ = Abundance/Large presence

Table 1. Phytochemical constituents of *Eugenia uniflora* .L Leaves.

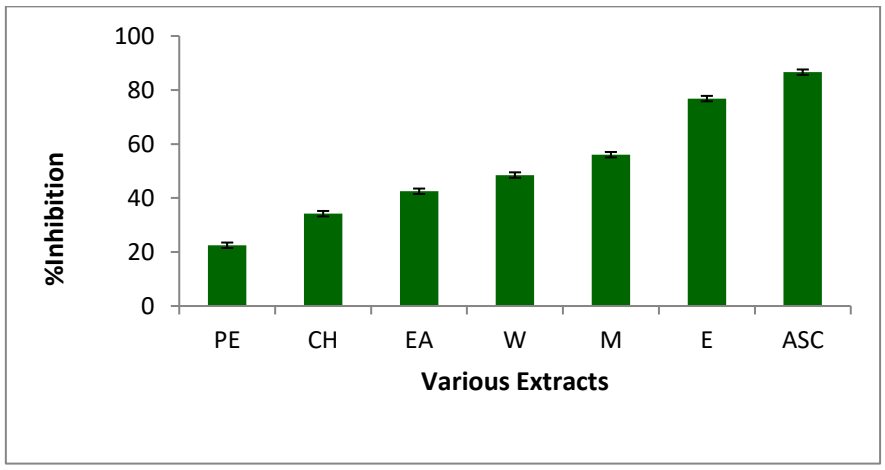

Fig.1. DPPH activity of various extracts of *Eugenia uniflora* L. PE =Petroleum ether, CH= chloroform, EA =Ethyl acetate, W =water, M =methanol, E=Ethanol, ASC=Ascorbic acid.

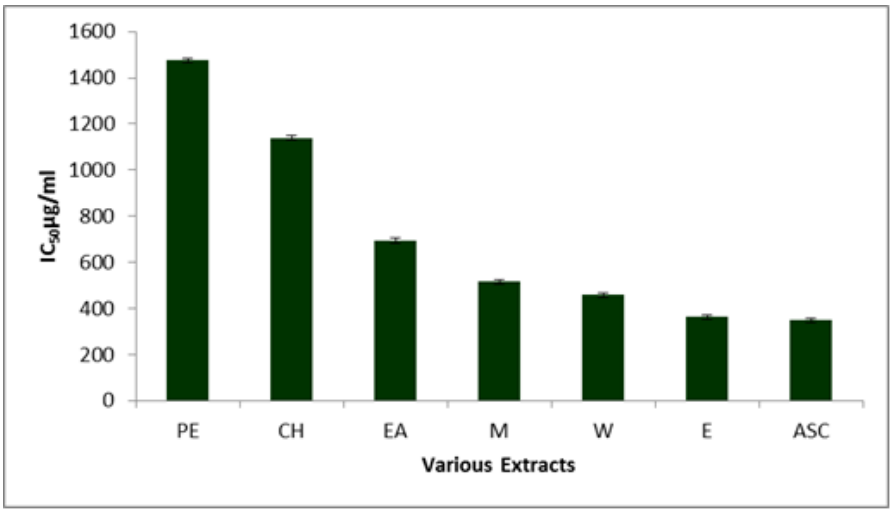

Fig 2. IC_{50} graph of DPPH activity of various extracts of *Eugenia uniflora* L. PE =Petroleum ether, CH= chloroform, EA =Ethyl acetate, W =water, M =methanol, E=Ethanol, ASC=Ascorbic acid.

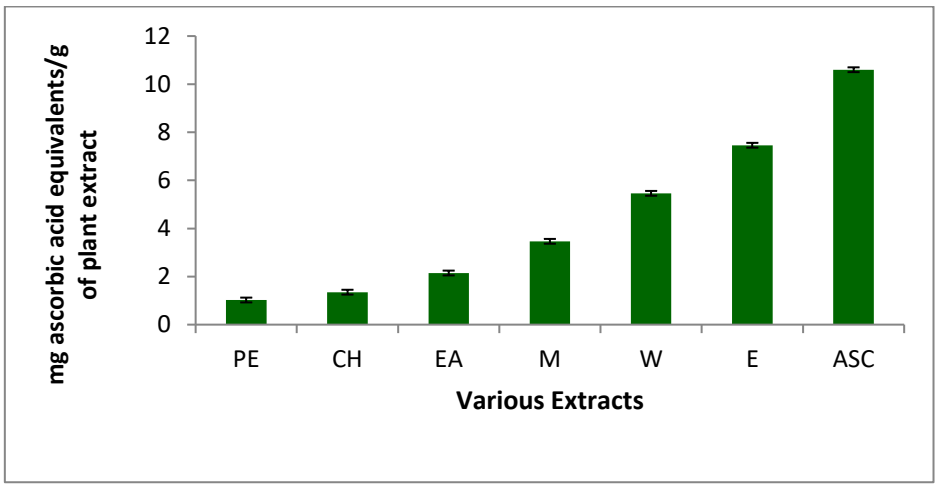

Fig.3. FRAP (Ferric Reducing Antioxidant Power) Assay.PE =Petroleum ether, CH= chloroform, EA =Ethyl acetate, W =water, M =methanol, E=Ethanol, ASC=Ascorbic acid.

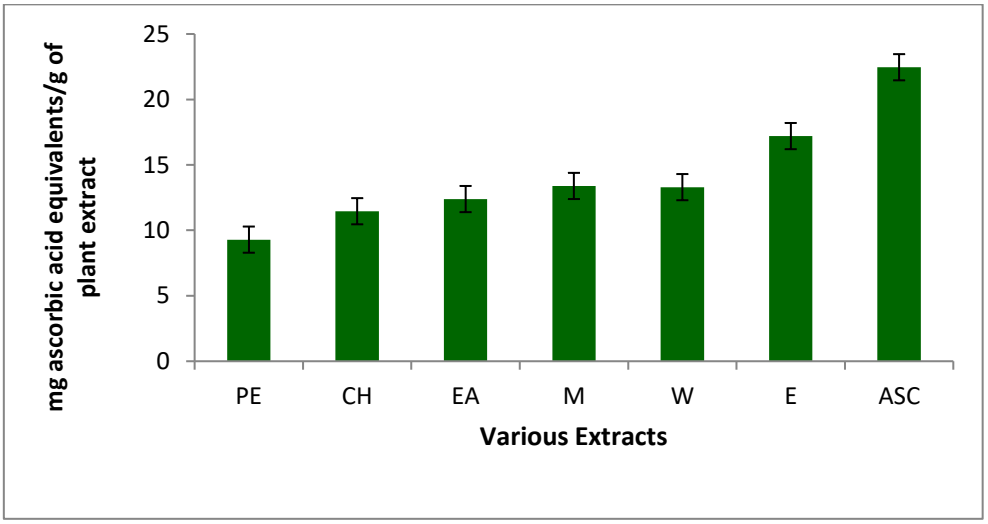

Fig.4. Total Antioxidant assay. PE =Petroleum ether, CH= chloroform, EA =Ethyl acetate, W =water, M =methanol, E=Ethanol, ASC=Ascorbic acid.

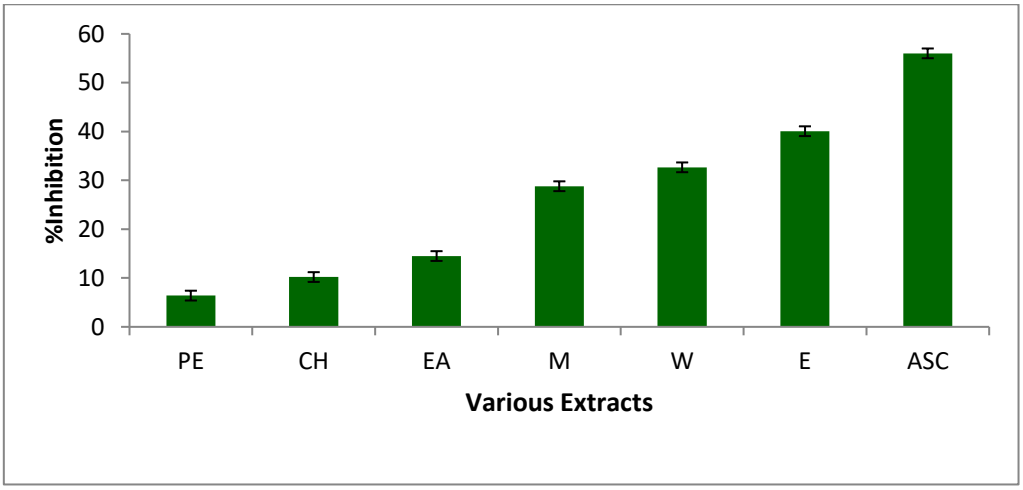

Fig.5. ABTS (2,2'-azino-bis 3-ethylbenzothiazoline -6-sulfonic acid) Assay .PE =Petroleum ether, CH= chloroform, EA =Ethyl acetate, W =water, M =methanol, E =Ethanol, ASC=Ascorbic acid.

CONCLUSION

The results of the study indicated that *Eugenia uniflora* is a potentially active plant and the ethanol extract of *Eugenia uniflora* are rich in phenolic contents and this extract exhibited strong antioxidant property. The free radical scavenging activities observed in DPPH, FRAPS, Total antioxidant assay and ABTS assays leads us to confirm *Eugenia uniflora* as a good source of natural antioxidants. Thus, it can be applicable in nutritional and pharmaceutical industry for developing new drugs against free radical mediated diseases. From this it can be concluded that *Eugenia uniflora* can be used as an additive in food industry in order to prevent oxidative damage.

REFERENCES:

1. Bagetti M, Facco E M Pesamosca, piccolo J, Amaya G E H D rodrieguez; Physciochemical characterization and antioxidants capacity of pitanga fruits (*Eugenia uniflora* L.) Food Science and Technology, 31(1):147-154. (2011).
2. Bakr R O, Mohamed S A, Waly N E; Phytochemical and biological investigation of *Eugenia uniflora* L. cultivated in Egypt. Journal of Pharmacognosy and Phytotherapy, Vol. 9(5), pp. 57-66, May 2017.gy Cienc. Tecnol. Aliment. vol.31 no.1 Campinas Jan./Mar. 2011.
3. Correa H A Martinez, Magalhaes P M, Quieroga C L, Peixoto C A;Extracts from pitanga (*Eugenia uniflora* L.) leaves :Influence of extraction process on antioxidant properties and yield of phenolic

compounds .j. of Supercritical Fluids 55,998-1006, 2011.

4. Countinho H D, Costa J G Martins, Jr J P Siqueira and Lima E Oliveira. Invitro screening by phototoxic properties of *Eugenia uniflora* L., Momordica charantia L., Mentha arvensis L. and Turnera ulmifolia L. Brazilian journal of Biosciences. R. bras. Bioci., Porto Alegree, v.8, n.3, p.299-301, jul./set.2010.
5. Daniel G. and Krishnakumari S; In vitro free radical scavenging activity of aqueous extract of *Eugenia uniflora*(L .) leaves .Journal of Medicinal plant studies: 4(4):25-29. 2016.
6. Daniel G., Krishnakumari S., Screening of *Eugenia uniflora* (L.) Leaves in Various Solvents for Qualitative Phytochemical Constituents. International journal of pharma and Biosciences 6(1):(B)1008-1015,2015.
7. Daniel G. and Krishnakumari S; Quantitative analysis of primary and secondary metabolites in aqueous hot extract of *Eugenia uniflora* (L.) Leaves. Asian journal of Pharmaceutical and Clinical Research, vol 8, issue 1,2015.
8. Denardin., Hirsch. C.C., Rocha. G.E., Vizzotto. R.F., Henriques. M., Moreira. A.T., Guma. J.C.F., F.T.C.R. &, T. Emanuelli. Antioxidant capacity and bioactive compounds of four Brazilian native fruits. Journal of Food and Drug Analysis, 23(3): 387-398. (2015).
9. Esene O, Uchechukwu R, Husseini S J, Thomas; Proximate and phytochemical analysis of leaf, stem, and root of *Eugenia uniflora* (Surinam or pitanga cherry).J.Nat.Prod.Plant Resour.,1(4):1-4. 2011.
10. Gacche R.N, Dhole N.A, Jadhav A.D.Free radical scavenging activity ,antioxidative ingredients, and possible anti –inflammatory abilities of selected traditional medicinal plants from Ayurveda. Journal of Natural Remedies. Vol. 10/1 64 - 73(2010).
11. Gujjeti R.P and Mamidala E. Phytochemical screening and thin layer chromatographic studies of Aerva Lanata Root extract. International Journal of Innovative Research in Science, Engineering and Technology. Volume 2, Issue 10, October 2013.
12. Facco M B., P.E.M.P., Hirsch. J., Amaya. G.E.R., C.N. D. Kobori., T. Emanuelli. Physicochemical characterization and antioxidant capacity of pitanga fruits (*Eugenia uniflora* L.). Food Science and Technology, 31(1):147-154. (2011).
13. Figueiroa E O., Silva L.C.N., Melo C.M., Neves. J.K. A Lemonie., Silva N.H., Pereira. VR.A., Correia. M.T. Santos. Evaluation of Antioxidant, Immunomodulatory, and Cytotoxic Action of Fractions from *Eugenia uniflora* L. and Eugenia malaccensis L.: Correlation with polyphenol and flavonoid content. The scientific world journal, Article Id 125027,7pages Volume 2013.
14. Malaman F.S., Moraes L.A.B., West.C., Ferreira.N.J., Oliveira.A.L.,Supercritical fluid extracts from the Brazilian cherry (*Eugenia uniflora* L.):Relationship between the compounds and the characteristics flavour intensity of the fruit. Food chemistry 124 (2011)85-92.
15. M.Govindappa., S.N. Sravya., M.N. Poojashri., T.S. Sadananda and C.P. Chandrappa. Antimicrobial, antioxidant and in vitro anti-inflammatory activity of ethanol extract and active phytochemical screening of Wedelia trilobata (L.) Hitchc. Journal of pharmacognosy and Phytotheraphy Vol.3(3) pp.43-51, April 2011.
16. Mohanapriya., C,Uma .S., Modilal.R.D., Nithyalakshmi. V., Phytochemical screening and invitro antioxidant studies on acetone extract of Manilkara Zapota L. seeds. International Journal Pharmaceutical Sciences Research (IJPSR), vol, 5(6):2354-61, (2014).
17. Misbah H., Aziz A Abdul.,and Aminudin N.,Antidiabetic and antioxidant properties of Ficus deltoidea fruit extracts and fractions. Misbah et al. BMC Complementary and Alternative Medicine,13:118,2013.
18. Pallab K, K B Tapan,K P Tapas, Ramen K .Estimation of total flavanoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum Sensitivum Linn*. Journal of Drug Delivery & Therapeutics; 3(4), 33-37 33 ,2013.
19. Rahman Md Mostafizur, Rumzhum N N, Zinna K-E-Khuda. Evaluation of Antioxidant and antinociceptive properties of methanolic extract of *Clerodendrum Viscosum Vent.* S. J. Pharm. Sci. 4(1): 74-78 74 S. J. Pharm. Sci. 4(1): 74-78.,2011.
20. Rajurkar N S and Hande S M. Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants, Indian J Pharm Sci, 73 (2): 146-151, 2011.
21. Reynertson K A, Basile M J and Kennelly E J; Antioxidant potential of seven myrtaceous fruits.ethnobotanyjournal.org/vol3/i1547-3465-03-025.pdf.
22. Santos R.M, Fortes G.A.C, Ferri P H, Santos S C ; Influence of foliar nutrients on phenol levels of *Eugenia uniflora*. Brazilian journal of pharmacognosy,21(4),581-586, ju/Aug 2011.
23. N O. Nwanneka, U N Obioma, Elijah J P, Joseph O C. Department of Biochemistry, University of Nigeria. Antioxidant effect of *Eugenia uniflora* pulp extract on the pro-and antioxidant status of carbon tetrachloride (CCl4) –induced hepatotoxicity in Albino rats. International journal of current Biomedical and pharmaceutical research. Int J cur Biomed Phar Res.2011:1(4):157-163.
24. NS Schumacher., TC Colomeu., D Figueiredo., V C Carvalho., CB Cazarin ., MA Prado., LM Meletti., R L Zollner .Identification and antioxidant activity of the extracts of *Eugenia uniflora* leaves. Characterization of the anti-inflammatory properties of Aqueous extract on Diabetes Expression in an Experimental Model of spontaneous Type 1 Diabetes (NOD Mice) Antioxidants (Basel). Oct 9.4(4):662-80. Doi:10.3390/antiox4040662. 2015.