IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
August 2016 Vol.:4, Issue:2

Analysis of Flavanoids in Aqueous Extract of *Eugenia uniflora* (L.) Leaves Using HPTLC

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Geedhu Daniel and S.Krishnakumari

Department of Biochemistry,Kongunadu Arts and Science College,Coimbatore,Tamilnadu,India-641029

Submission: 5 August 2016
Accepted: 10 August 2016
Published: 25 August 2016

Keywords: HPTLC, Flavonoids, *Eugenia uniflora*

ABSTRACT

Most of the traditional medicinal plants in India are not scientifically validated. Scientific evaluation along with traditional knowledge is essential to obtain effective drugs for commercial purpose. The aim of the study was to establish the chemical fingerprint of flavonoid profile in aqueous extract of *Eugenia uniflora* leaves. Fingerprint analysis by HPTLC has become an effective and powerful tool for linking the chemical constituent profile of the plants with botanical identity and for the estimation of chemical and biochemical markers. HPTLC analysis was done and profiles were developed for authentication. The aqueous extract of *Eugenia uniflora* showed the presence of several flavonoids. The development of such fingerprint for leaves is useful in differentiating the species from the adulterant and also acts as biomarker for this plant in pharmaceutical industry.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [1]. Many of these indigenous medicinal plants are used as spices and food plants. Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents.

Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases [2-4]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [5].

It has been shown that *in-vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [6].Modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbals and its formulations. Also, the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled techniques and applying suitable standards [7, 8] .

Modern high-performance TLC (HPTLC) is an efficient instrumental analysis, and optimized quantitative HPTLC using a densitometric evaluation can produce results analogous to those obtained with gas chromatography (GC) and high performance liquid chromatography (HPLC) [9,10]. Thus, HPTLC ‘fingerprint analysis’ may be a powerful tool for the quality control of raw plant material and may be an alternative technique, particularly in the analysis of crude plant extracts. An improvement over conventional TLC, HPTLC is an instrumental technique whereby special plates and instrumental resources for sampling are used and the quantitative evaluation of

separations is aided by densitometry [11]. HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter duration[12].

Eugenia uniflora L. (Myrtaceae) is a tropical and subtropical shrub widely distributed in American countries [13]. It is commonly referred to as Pitanga cherry or Brazilian cherry. Regarding their effects on human health, both fruit and leaves are used as folk medicine to treat similar diseases, although the leaves show the advantage of being perennial and continuously available, while the fruits are available during a short period of the year [14]. The fresh or dried leaves have been used empirically as medicine, since the 15th century, for treating inflammatory and stomach diseases, rheumatism, fever, and hypertension [15, 16]. Some studies have confirmed that *Eugenia uniflora* possesses anti-inflammatory, antimicrobial, and antifungal properties [15, 17–19]. These benefits are usually attributed to the presence of many secondary metabolites present in the leaves, which includes many volatile terpenoid oils, flavonoids, and condensed and hydrolysable tannins, leucoanthocyanidins, and steroids and/or triterpenoids [20].

Flavonoids are present in many plant extracts, being constantly the focus of pharmacological studies. Despite their well-described antioxidant activity [21-23], they have shown many other properties, such as anti-inflammatory, antimicrobial, antiaging, anticancer, and antiallergic, hypocholesterolemic and vasodilatation [21-26]. Together, the anti-inflammatory and antioxidant properties of flavonoids can explain the efficacy of plant extracts against various diseases, such as osteoporosis and rheumatism [27]. Therapeutic properties attributed to the flavonoid contents of *E. uniflora* against inflammatory disorders were reported.

With this knowledge, the present research work was aimed to produce the fingerprint analysis using HPTLC to profile flavonoids in leaf extract of *Eugenia uniflora.*

MATERIALS AND METHODS

Plant Collection and Authentication

Fresh leaves of *Eugenia uniflora (*Linn), Family- Myrtaceae, were collected from Wayanad district, Kerala during the month of April 2014. Taxonomic authentication was done by Dr. V.S Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India.

Sample Processing

The leaves were washed, shade dried at room temperature and powered in a mixer grinder.

Extraction Procedure:

Author Please add all this

HPTLC Analysis in Aqueous Extract of *Eugenia uniflora* for Flavonoid Profile

Flavonoids are important components of 'functional foods', with beneficial effects on the cardiovascular function, mainly due to their antioxidant activity. Many flavonoids exert antihypertensive, anti-atherosclerotic and antiplatelet activity and positive effects against endothelial dysfunction. Recent evidence indicates that they exert cardioprotective effects against myocardial ischaemia/reperfusion (I/R) injury. The aim of this work was to investigate these properties for flavonoids with different structural characteristics.

Procedure

The aqueous extract of *E.uniflora* leaves was centrifuged at 3000 rpm for 5 min. This solution was used as test solution for HPTLC analysis 0.5 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using hamilton syringe and Camag Linomat 5 instrument. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapor) with respective mobile phase (flavonoid) and the plate was developed in the respective mobile phase (ethyl acetate-butanone-formic acid-water (5:3:1:1) up to 90mm.The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (camag reprostar 3) and captured the images at visible light, UV 254 nm and UV366 nm.The developed plate was sprayed with respective spray reagent (flavonoid) (1% ethanolic aluminium chloride reagent) and dried at 100^0C in hot air oven. The plate was photo-documented in visible light and UV 366 nm mode using photo-documentation (camag reprostar 3) chamber. After derivatization, the plate was fixed in scanner stage (camag tlc scanner 3) and scanning was done at UV 366 nm. The peak table, peak display and peak densitogram were noted. The software used was win CATS 1.3.4 version.

Detection

Yellow, yellowish blue coloured fluorescent zone at UV 366 nm mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of flavonoids in the given standard and in the sample.

RESULTS

Herbal medicines are composed of many constituents and are therefore very capable of variation. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active components of the herbal medicine. HPTLC fingerprinting profile is very important parameter of herbal drug standardization for the proper identification of medicinal plants.

The present study was oriented towards the flavonoid profile screening of the species, *Eugenia uniflora* and development of fingerprints using HPTLC technique. The HPTLC chromatogram

can be best observed under fluorescence 254 nm &366 nm before and after derivatization. Several bands of flavonoids are seen to be separated before derivatization at 366 nm. The major compounds separated were seen at Rf = 0.52, 0.69 & 0.79. Best solvent system to observe the above separation is, ethyl acetate-butanone-formic acid-water (5:3:1:1).The result is given in Table 1, Figure 1 & 2.

Table 1: Peak table with Rf values for HPTLC analysis of aqueous extract of *Eugenia uniflora leaves*

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.31	594.1	21477.2	Flavonoid standard
Sample I	1	0.06	391.0	10822.8	Unknown
Sample I	2	0.15	168.1	6929.5	Unknown
Sample I	3	0.20	135.5	9061.0	Unknown
Sample I	4	0.52	53.4	1893.0	Flavonoid 1
Sample I	5	0.55	51.6	1615.2	Unknown
Sample I	6	0.69	202.2	9438.8	Flavonoid 2
Sample I	7	0.79	35.7	1635.0	Flavonoid 3
Sample I	8	0.99	14.8	100.5	Unknown

* *Sample I- Aqueous extract of Eugenia uniflora leaves*

Before derivatization

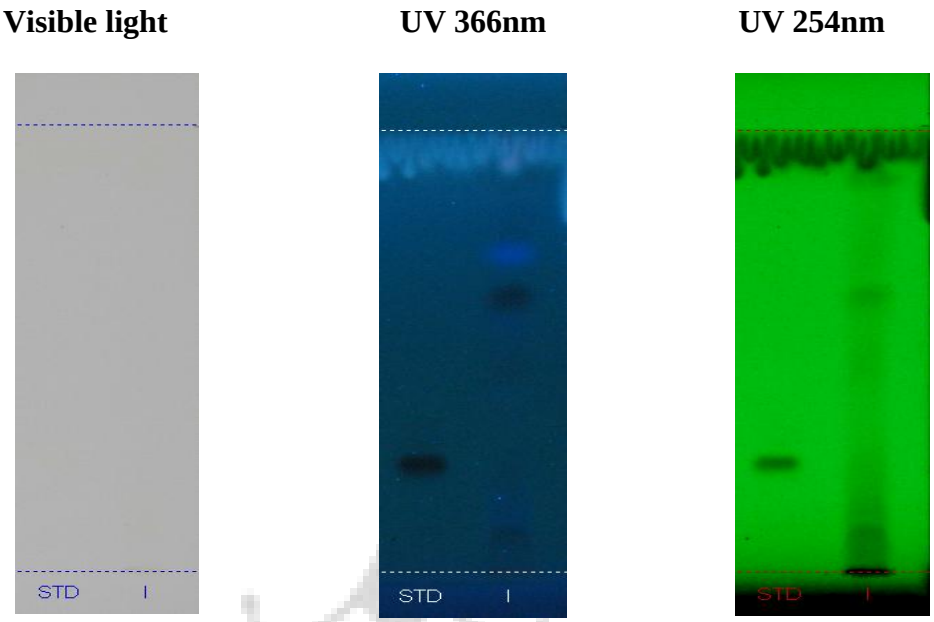


Figure 1: Chromatogram results for flavonoids in HPTLC analysis before and after derivatization under visible and UV light.

After derivatization

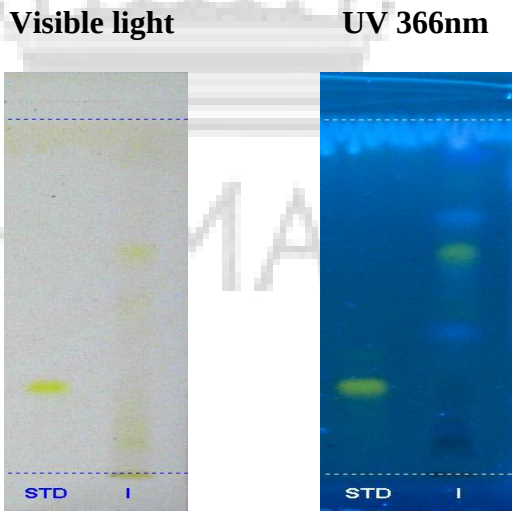
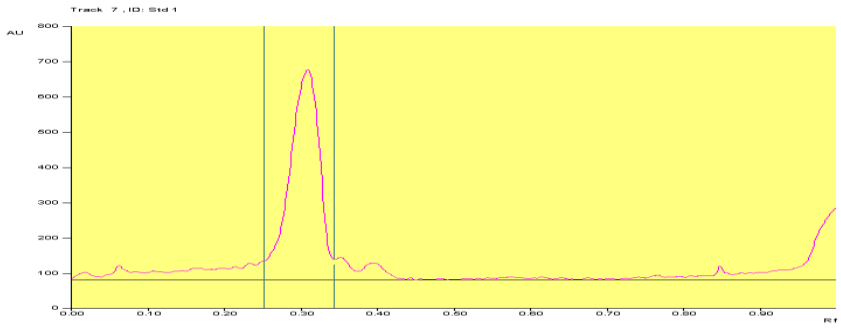


Figure 2: Baseline and densitogram for flavonoids standard

Track STD – Flavonoid standard Baseline display (Scanned at 366nm)



Track STD – Flavonoid standard Peak densitogram display (Scanned at 366nm)

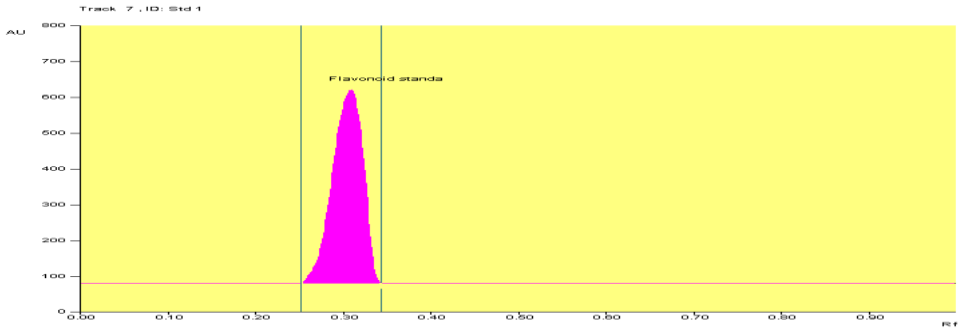
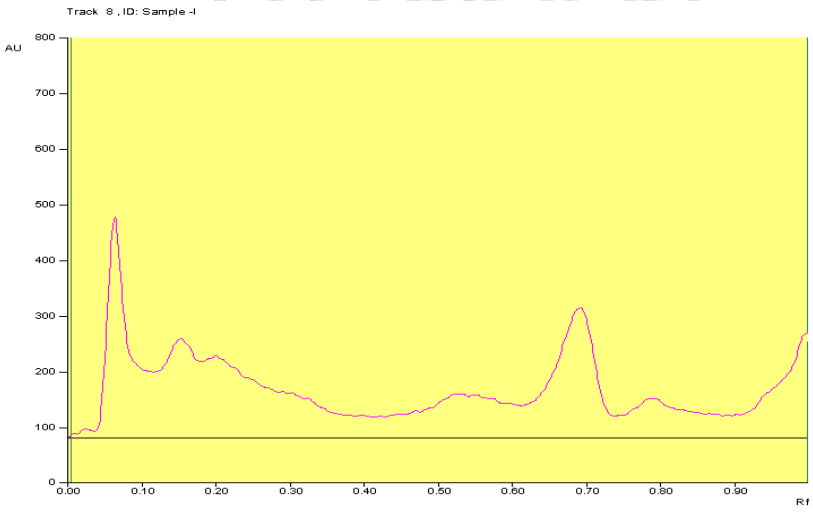
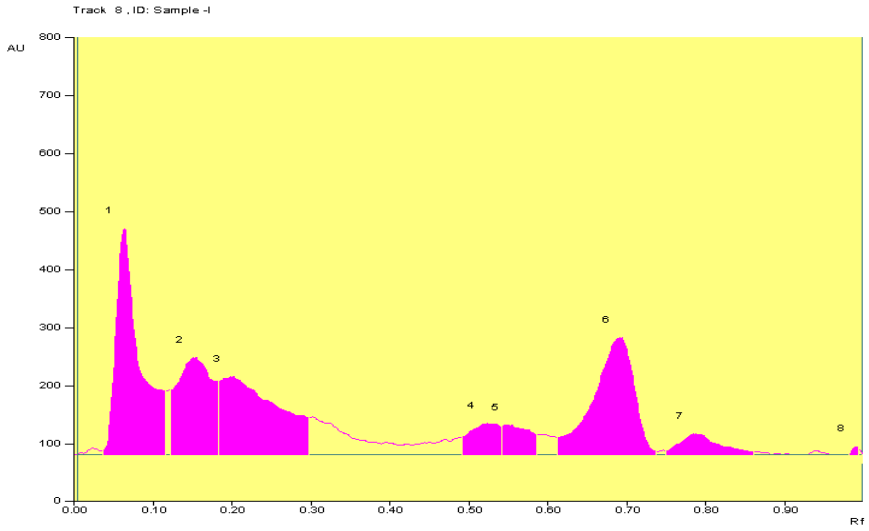


Figure 3: Baseline and Densitogram for flavonoids in aqueous extract of *Eugenia uniflora* leaves

Track I –Aqueous extract of *Eugenia uniflora*Baseline display (Scanned at 366nm)



Track I –Aqueous extract of *Eugenia uniflora* Peak densitogram display (Scanned at 366nm)



DISCUSSION

Nature produces a tremendous array of secondary metabolites or natural products with most diversity seen in microorganisms and plants [28, 29]. Natural products are the main sources of bioactive molecules and have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases [30]. Among the bioactive secondary metabolites present in medicinal plants alkaloids, flavonoids, isoprenoids are of high interest. Humans exploit natural products as a source of drugs, flavoring agents, fragrances and for a wide range of other applications.

Several methods available for separating plant constituents, the chromatographic procedure is the most commonly used techniques for general application. The present HPTLC studies confirmed the presence of active metabolite in the aqueous extract of *Eugenia uniflora* leaves. A good separation of flavonoids has been observed in present HPTLC analysis.

Flavonoids are plant pigments that are synthesized from phenylalanine, generally display marvelous colors known from flower petals, mostly emit brilliant fluorescence when they are excited by UV light and are ubiquitous to green plant cells. It inhibits many bacterial strains, inhibits important viral enzymes, such as reverse transcriptase and protease, and destroy some pathogenic protozoans. It has ability to inhibit specific enzymes, to stimulate some hormones and act as neurotransmitters [31]. Most flavonoids function in the human body as antioxidants [32]

and control inflammation [33]. Flavonoids are reported to have anti-oxidant anticancer anti-allergic, anti-inflammatory, anti-carcinogenic and gastroprotective properties. Common examples for flavonoids are quercetin, rutin, hesperidin etc. The aqueous extract of *Eugenia uniflora* leaves revealed 3 different types of flavonoids with different Rf levels.

CONCLUSION

Standardization of plant materials is the need of the day. An HPTLC fingerprint is suitable for rapid and simple authentication. The HPTLC fingerprint developed may serve as a supplement chromatographic data and the information thus generated may be explored further as a tool for standardization. HPTLC analysis revealed a better separation of secondary metabolites, flavonoid.

The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hitherto unmet therapeutic needs. These plant derived bioactive compounds in addition of being developed directly as drugs can also serve as prototype drug molecules known as "Lead Compounds" and as pharmacological probes to help better understand biochemical and physiological mechanisms [34]. Bioactivity guided fractionation can lead to the isolation of active principle of this plant and some of the chemical entities with acceptable pharmaceutical qualities can be developed as drugs in their original form directly. In addition to their medicinal use some secondary metabolites from these plants can also serve as powerful "pharmacological tool" to help explain the mechanism underlying human diseases [35,36].

REFERENCES

1. Garro L.C., Intercultural variation folk medicinal knowledge: A comparison between curers and noncurers,American anthropologist, 1986, 88, 351-370
2. Eisenhauer N., Klier M., Partsch S., Sabais A.C.W., Scherber C., Weisser W. and Scheu S., No interactiveeffects of pesticides and plant diversity on soil microbial biomass and respiration, *Appl. Soil Ecol*., 2009,42, 31-36.
3. Uzer A., Ercag E. and Apak R., Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction, *Anal.Chim.Acta,* 2005,534, 307-317.
4. Pico Y. and Kozmutza C., Evaluation of pesticide residue in grape juices and the effect of natural antioxidants on their degradation rate, *Anal.Bioanal.Chem.*,2007, 389,1805-1814.
5. Khalaf N.A., Shakya A.K., Al-othman A., Ahbar Z. and Farah H., Antioxdidant activity of some common plants,*Turk. J. Biol*.,2007, 31, 1-5.

6. Mathekaga A.D. and Meyer J.J.M., Antibacterial activity of South African *Helichrysum*species, *South Afr. J. Bot*.,1998,64, 293-295.
7. Okwu, D.E.The Potentials of *Ocimumgratissimum*, *Penghuriaextensa*and *Tetrapleureatetraptera*as spice and flavouring Agents. 2006 J. Chem. Soc. Nigeria, 31(1, 2): 38-42.
8. Mathekaga AD, Meyer JJM. Antibacterial activity of South African*Helichrysum*species. South Afr. J. Bot. 1998; 64:293-295.
9. Wagner H, Bladt S. Plant Drug Analysis. A Thin Layer Chromatography Atlas. Berlin : Springer; 2001.
10. Medic-Saric M, Jasprica I, Mornar A, Males Z. Thin Layer Chromatography in Phytochemistry. 2nd ed. CRC, Press; 2008. Application of TLC in the isolation and analysis of flavonoids.
11. Nile SH, Park SW. HPTLC analysis, antioxidant, anti-inflammatory and antiproliferative activities of Arisaematortuosum tuber extract. Pharma. Biol. 2014;52:221–227.
12. NadkarniA.K.Indian Materia Medica, Vol. 1, Popular Prakashan, Bombay. 2005;972-973
13. A. D Rotman, "Las especiesargentinas del genero*Eugenia* L. (Myrtaceae)," *Boletin de la Sociedad Argentina de Botanica*,1995, vol. 30, pp. 63–93.
14. A. Kanazawa, A. Patin, and A. E. Greene, "Efficient, highly enantioselective synthesis of selina-1,3,7(11)-trien-8-one, a major component of the essential oil of *Eugenia uniflora*," *Journal ofNatural Products*, 2000,vol. 63, no. 9, pp. 1292–1294.
15. J. R. Alonso, *Tratado de Fitomedicina*, Isis Ediciones S.R.L, Buenos Aires, Argentina, 1998.
16. A. C. Adebajo, K. J. Oloke, and A. J. Aladesanmi, "Antimicrobial activities and microbial transformation of volatile oils of *Eugenia uniflora*," *Fitoterapia*, 1989,vol. 60, no. 5, pp. 451–455.
17. A. E. Consolini and M. G. Sarubbio, "Pharmacological effects of *Eugenia uniflora* (Myrtaceae) aqueous crude extract on rat's heart," *Journal of Ethnopharmacology*,2002, vol. 81, no. 1, pp. 57–63.
18. E. E. S. Schapoval, S. M. Silveira,M. L.Miranda, C. B. Alice, and A. T. Henriques, "Evaluation of some pharmacological activities of *Eugenia uniflora*L," *Journal of Ethnopharmacology*, 1994,vol. 44, no. 3, pp. 137–142.
19. F. B. Holetz, G. L. Pessini, N. R. Sanches, D. A. G. Cortez, C. V. Nakamura, and B. P. Dias Filho, "Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases," *Memorias do Instituto Oswaldo Cruz*, 2002,vol. 97, no. 7, pp. 1027–1031.
20. E. O. Lima, O. F. Gompertz, A. M. Giesbrecht, and M. Q. Paulo, "In vitro antifungal activity of essential oils obtained from officinal plants against dermatophytes," *Mycoses*, 1993,vol. 36, no. 9-10, pp. 333–336.
21. A. C. L. Amorim, C. K. F. Lima, A. M. C. Hovell, A. L. P. Miranda, and C. M. Rezende, "Antinociceptive and hypothermic evaluation of the leaf essential oil and isolated terpenoids from *Eugenia uniflora*L. (Brazilian Pitanga)," *Phytomedicine*, 2009vol. 16, no. 10, pp. 923–928.
22. F. Cacciola, P. Jandera, Z. Hajdu´ , P. C˘ esla, and L. Mondello, "Comprehensive two-dimensional liquid chromatography with parallel gradients for separation of phenolic and flavone antioxidants," *Journal of Chromatography A*,2007, vol. 1149, no. 1, pp. 73–87.
23. A. Gugliucci, "Antioxidant effects of Ilex paraguariensis: induction of decreased oxidability of human LDL in vivo," *Biochemical and Biophysical Research Communications*, 1996,vol. 224, no. 2, pp. 338–344.
24. N. Dartora, L. M. De Souza, A. P. Santana-Filho, M. Iacomini, P. A. J. Gorin, and G. L. Sassaki, "UPLC-PDA-MS evaluation of bioactive compounds from leaves of Ilex paraguariensis with different growth conditions, treatments and ageing," *FoodChemistry*, 2011,vol. 129, no. 4, pp. 1453–1461.
25. M. Sumino, Y. Saito, F. Ikegami, Y. Hirasaki, and T. Namiki, "Extraction efficiency of shosaikoto (Xiaochaihu Tang) and investigation of the major constituents in the residual crude drugs," *Evidence-Based Complementary and AlternativeMedicine*, vol. 2012, Article ID 890524.
26. A.-R. Im, Y.-H. Kim, M. R. Uddin et al., "Scutellariabaicalensis extracts and flavonoids protect rat l6 cells from antimycin a-induced mitochondrial dysfunction," *Evidence-Based Complementary and Alternative Medicine*, vol. 2012, Article ID 517965.
27. M. A. AbdJalil, A. N. Shuid, and N. Muhammad, "Role of medicinal plants and natural products on osteoporotic fracture healing," *Evidence-Based Complementary and AlternativeMedicine*, vol. 2012, Article ID 714512.

28. Wink M; Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective; Phytochemistry; 2003; 64: 3-19
29. Konig, G.M, Kehraus S, Seibert SF, Abdel-Lateff A and Muller D; Natural products from marine organisms and their associated microbes; Chembiochem; 2006; 7:229-238
30. Newman DJ, Cragg GM; Natural products as sources of new drugs over the last 25 year Natprod, 2007; 70:461-77
31. Havsteen BH. The biochemistry and medical significance of the flavonoids. PharmacolTher 2002; 96:67-202.
32. Gil MI, Ferreres F, Tomas-Barberan FA. Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. J Agric Foo25. Havsteen BH. The biochemistry and medical significance of the flavonoids. PharmacolTher 2002; 96:67-202.
33. Panthong A, Kanjanapothi D, Tuntiwachwuttikul P, Pancharoen O, Reutrakul V. Anti-inflammatory activity of flavonoids. Phytomedicine 1994; 1:141-144.d Chem 1999; 47:2213-2217.
34. Osbourn AE, Lanzotti V; Plant derived Natural Products, Synthesis Function and application; Springer Science; 2009
35. Newman DJ, Cragg GM, Holbeck S, Sansville EA. Natural products and derivatives as leads to cell cycle pathway targets in cancer chemotherapy; Current cancer drug targets; 2002; 2; 279-308
36. Koehn FE. Therapeutic potential pf Natural product signal transduction agent; Current OpinBiotechnol; 2006; 17; 631-637.