A Study on Trans-Resveratrol and Umbelliferone in *Averrhoa bilimbi* Linn. FRUITS

Chinju Merin Abraham   Lecturer in Botany, Department of Botany, Catholicate College, Pathanamthitta

ABSTRACT   *Averrhoa bilimbi, L. (Oxalidaceae), the widely cultivated plant among tropical countries was studied for Trans-resveratrol and Umbelliferone. The computer aided system PASS (Predication of Activity Spectra for Substances) was used for the analysis of pharmaco-therapeutic potential of various constituents. Numerous potentials of the fruit was revealed by the PASS analysis. The quantification of Trans-resveratrol and Umbelliferone from A. bilimbi fruit using HPLC has resulted in 7 peaks. Of these, two peaks were identified as Trans-resveratrol and Umbelliferone, which was resolved at retention time (Rt) 35.02 and 18.26 respectively. More in vitro and in vivo studies together with epidemiological and clinical-case control studies are needed before recommending A. bilimbi fruits as functional food.*

INTRODUCTION

Traditional medicines are still the mainstay for treating various diseases across the world. The family Oxalidaceae, or 'wood sorrel family' typically have divided leaves, the leaflets showing "sleep movements", spreading open in light and closing in darkness. The use of Oxalidaceae family members for the treatment of various ailments are recorded from time immemorial. *A. bilimbi* fruits are administered as a treatment for cough, beri-beri and biliousness. The fruit Syrup is used to cure fever and inflammation and to stop rectal bleeding and alleviate internal hemorrhoids by traditional healers (Ali et al., 2013). Moreover, Bilimbi fruit juices are used for cleaning pots and also to remove iron rust from metals and to bleach stains from the hands. The antihyperlipidaemic (Ambili et al., 2009); antimicrobial, cytotoxic, antithrombotic and antioxidant activities (Kumar et al., 2013) of the fruits of *A. bilimbi* was well reported from various regions.   However no work has been carried out on the fruits of the plant for analyzing the Trans-resveratrol and Umbelliferone components and hence the present study.

MATERIALS AND METHODS

Plant material

Fresh bilimbi fruits were collected from Central Travancore region and the collected fruits were washed under running tap water followed by distilled water, dried and powdered.

Systematic position

Kingdom : Plantae

Division : Magnoliophyta

Class   : Dicotyledonae

Subclass : Rosidae

Order   : Oxalidales

Family   : Oxalidaceae

Genus   : *Averrhoa*

Species   : *blimbi L.*

Liquid Chromatography - Mass spectrometry (LC-MS) analytical technique was used for identifying various phytochemicals in the extract (Lim and Lord, 2002).

PASS analysis

This computer system can predict biological activity based on structural formula of a chemical compound. The PASS approach is based on the suggestion, Activity = Function (Structure). Thus, "comparing" structure of a new substance with that of the standard biologically active substances, it is possible to find out whether a new substance has a particular effect or not. PASS estimates the probabilities of particular substances belonging to the active and inactive sub-sets from the SARBase (Structure-Activity Relationships Base) (Gloriozova et al., 1998).

Resveratrol & Umbelliferone – HPLC Profiling

Standard Trans-resveratrol and Umbelliferone were used for the comparison with the test material. High Performance Liquid Chromatography (Shimadzu HPLC class VP series) with PDA (Photodiode Array) detector was used. The column used was reverse phase (RP) Phenomenex-C-13 column with 25cm length and 5mm diameter. Solvent system used was Methanol and Acetonitrile in the ratio of 1:1. Reverse phase HPLC was performed with an isocratic elution and the injection volume was 20μl. The obtained results were compared with standard too.

The method used for the quantification was area under curve method, and equation used for quantification was:

$$A = X \times Y \times P \div Z \times S$$

Where, A = Percentage of marker compound in the drug

X = Concentration of standard marker injected

Y = Area given by the marker compound in the sample profile

P = Purity of the marker compound taken (99%)

Z = Area given by the standard compound

S = Sample concentration with respect to the drug taken

RESULTS

Results of LC-MS analysis of major classes of metabolites in bilimbi fruit and the integrated library search (METWIN 2.0) results with the list of compounds was given in Table 1. The computer aided system PASS (Predication of Activity Spectra for Substances) was used for the analysis of pharmaco-therapeutic potential of various constituents. In the present study, PASS analysis was conducted for Trans-Resveratrol and Umbelliferone identified through LC-MS - methanol fraction. The results of PASS analysis of Trans-Resveratrol and Umbelliferone from *A. bilimbi* fuits was given Table 2 and 3 respectively. The quantification of Trans-resveratrol and Umbelliferone from *A. bilimbi* fruit using HPLC has resulted in 7 peaks (Figure 1 and Table

4). Of these, two peaks were identified as Trans-resveratrol and Umbelliferone, which was resolved at retention time (Rt) 35.02 and 18.26 respectively. The result of quantitative estimation of Trans-resveratrol and Umbelliferone was given in Table 5.

DISCUSSION

The LC-MS analysis has recorded a total of 20 different compounds from the tested *A. bilimbi* fruits. It is well illustrated that *A. bilimbi* fruits are phytochemically rich with a myriad of biological effects. It can be concluded that the synergistic action of these phytochemicals are the reason behind the antihyperlipidaemic, antimicrobial, cytotoxic, antithrombotic, antioxidant activities and other diverse activities of the fruits of *A. bilimbi*. The fruits of *A. bilimbi* can be recommended as a nutraceutical functional food. Currently, mushrooming interest is witnessed in nutraceuticals which provide health benefits and are alternative to modern medicine (Chauhan et al., 2013). The presence of Trans-resveratrol (trans-3,4,5-trihydroxystilbene) and Umbelliferone (7-Hydroxycoumarin) were recorded in the study. The antioxidant, anti-inflammatory, anti-platelet, anti-allergic and anti-carcinogenic potential of Trans-resveratrol was already reported (Juan et al., 2005). Another compound, Umbelliferone is a widely occurring phenolic compound of plant origin which has proven free radical scavenging activity (Singh et al., 2010). The physiological, bacteriostatic and anti-tumor activity of these compounds makes them an attractive and potential candidate in developing novel therapeutic agents (Jain and Joshi, 2012).

The PASS analysis of the *A. bilimbi* fruit extract has resulted in a plethora of combinations with a multitude of biological activities like anticarcinogenic, antihelmintic, antihypoxic, antineoplastic, antiseborrheic, anti-apoptosis, capillary fragility treatment, cardioprotectant, carminative, cytoprotecnt, fibrinolytic, lipid metabolism regulator, mucomemberanous protector, neurotransmitter antagonist, sickle-cell anemia treatment and spasmolytic. The PASS can be predicted on the basis of structure-activity relationship found by the analysis of the known data from the training set which is the cornerstone for new noetic combinations to combat various ailments. It can be concluded that it is a potential source of high value components for pharmaceutical and nutraceutical industry. The HPLC analysis revealed that 7 peaks were detected and 2 peaks were identified as Trans-resveratrol and Umbelliferone was resolved at retention time (Rt) 35.02 and 18.26 respectively. The presence of Trans-resveratrol among different plants and plant products was observed through HPLC earlier like red wine (Suoto et al., 2001) and cocoa liquor (Counet et al., 2006).

A. bilimbi fruits are rich in phytochemicals which are biologically active and provide health benefits for humans. However in vitro and in vivo studies in animal models and via epidemiological and clinical-case control studies among human are mandatory.

CONCLUSION

Mother Nature is a unique source of structures of high phytochemical diversity with many of them possessing punctilious biological activities and medicinal attributes. *A. bilimbi* fruits was tested for Trans-resveratrol and Umbelliferone components via HPLC. The HPLC analysis revealed Trans-resveratrol and Umbelliferone was resolved at retention time (Rt) 35.02 and 18.26 respectively among A. bilimbi fruits.

Table 1: List of compounds from *A. bilimbi* fruit extracts by LC-MS

Sl. No.	Compound name	Molecular mass
1	Trans-resveratrol	228.5
2	Umbelliferone	161.12
3	Salicylic acid	138.12
4	Methyl salicylate	152.54
5	Dihydro myrecetin	320.26
6	Eriocitrin	596.22
7	Boswellic acid	456.54
8	Hydroxy aristolochic acid	357.28
9	Cinnnamaldehyde	132.08
10	Benzyl cinnamate	238.29
11	Hydroxy-citric acid lactone	190.11
12	Benzyl alcohol	108.14
13	Phenyethyll amine	121.16
14	Leaf Alcohol	100.16
15	Caffeolmalic acid	296.23
16	Citric acid	490.11
17	Tartaric acid	150.08
18	Ascorbic acid	176.13
19	Xylose	150.14
20	Tyrosine	181.19

Table 2: Some predicted biological activities for Trans-Resveratrol by PASS analysis

No.	Pa	Pi	Activity
	0.908	0.009	Mucomemberanous protector
	0.888	0.011	Antiseborrheic
	0.823	0.012	Myocardial ischemia treatment
	0.715	0.040	Sickle-cell anemia treatment
	0.772	0.019	Lipid metabolism regulator
	0.756	0.007	Cytoprotecnt
	0.761	0.017	Antineoplastic (colorectal cancer)
	0.763	0.037	Non mutagenic, Salmonella
	0.752	0.021	Fibrinolytic
	0.748	0.017	Apoptosis agonist
	0.714	0.005	Antihelmintic (Nematodes)
	0.711	0.007	Carminative
	0.709	0.022	Antihypoxic

Pa and Pi are the probabilities of belonging to the classes of active and inactive compounds respectively.

Table 3: Some predicted biological activities for Umbelliferone by PASS analysis

No	Pa	Pi	Activity
1	0.943	0.003	Cardiovascular analeptic
2	0.861	0.008	Myocardial ischemia treatment
3	0.871	0.018	Antiseborrheic
4	0.857	0.005	Vascular(periferal) disease treatment
5	0.811	0.012	Pulmonary hypertension treatment
6	0.738	0.003	Anticarcinogenic
7	0.720	0.021	Apoptosis agonist
8	0.705	0.021	Mucomembranous protector
9	0.717	0.057	Cardioprotectant
10	0.815	0.004	Neurotransmitter antagonist
11	0.794	0.005	Spasmolytic , Urinary
12	0.704	0.004	Capillary fragility treatment
13	0.702	0.011	Kinase inhibitor
14	0.721	0.035	Cathepsin G inhibitor

Pa and Pi are the probabilities of belonging to the classes of active and inactive compounds respectively.

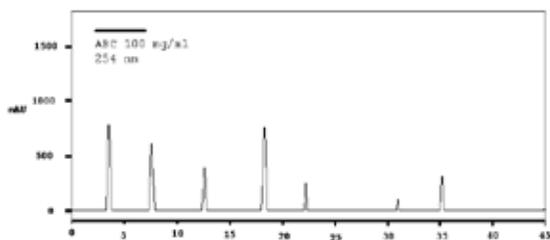**Fig 1: HPLC Chromatogram of *A. bilimbi* fruit extract**

Table 4: HPLC profile of *A. bilimbi* fruits

Peak Number	Retention Time in Min	Peak Area Obtained under the curve
1	4.10	1023
2	8.62	823
3	13.25	602
4	18.26 (Umbelliferone)	1112
5	22.65	402
6	30.05	101
7	35.02 (Trans-resveratrol)	420

Table 5: Quantification of compounds in *A. bilimbi* fruits by HPLC

Sl. No	Compounds	% (w/w)
1	Trans-resveratrol	0.328
2	Umbelliferone	1.397

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