



## A Study on Phytochemical Constituents of *Averrhoa Bilimbi* Linn. Fruits

### KEYWORDS

AVERRHOA BILIMBI, OXALIDACEAE, PHYTOCHEMISTRY, BILIMBI

### Chinju Merin Abraham

Lecturer in Botany, Department of Botany, Catholicate College, Pathanamthitta

**ABSTRACT** *Averrhoa bilimbi*, L. (*bilimbi*) (*Oxalidaceae*) is widely cultivated throughout tropical countries for their fruits. Thus a study was conducted in order to analyze the phytochemicals of *A. bilimbi* fruits. The presence of secondary metabolites especially flavonoids, Coumarins, Saponins and Phenols was reported. Methanol extract was more diverse (Flavonoids, Coumarins, Saponin, Phenols) followed by chloroform (Flavonoids, Coumarins, Phenols) and petroleum ether extracts (Saponin). The LC-MS analysis and the integrated library search results in the presence of 20 phytochemicals. Further works to separate the phytochemicals and testing individual mechanism of action was recommended

### INTRODUCTION

*Averrhoa bilimbi* commonly known as Bilimbi/Cucumber Tree belongs to family Oxalidaceae with ginormous medicinal value in alternative medicine to treat a plethora of ailments. The use of *A. bilimbi* for treating fever, mumps, pimples, inflammation of the rectum and diabetes, itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach ache and ulcer in folklore medicine by traditional healers are common (Anitha et al., 2011) as a part of their indigenous knowhow. *A. bilimbi* is a tropical tree, which prefers direct sunlight with evenly distributed rainfall throughout most of the year. The anti-diabetic, antimicrobial, anti-inflammatory, cyto-toxic, anti-oxidant and anti-fertility potentials of the plant were thoroughly investigated by many researchers (Tan et al., 2005; Zakaria et al., 2007; Savitri et al., 2009; Thamizhselvam et al., 2015).

### Systematic position

Kingdom : Plantae

Division : Magnoliophyta

Class : Dicotyledonae

Subclass : Rosidae

Order : Oxalidales

Family : Oxalidaceae

Genus : *Averrhoa*

Species : *bilimbi* L

*Bilimbi* has a short trunk dividing in to number of upright branches. Leaves are mainly clustered at the branch tips, are alternate, exstipulate, pinnate with a terminal leaflet. Flowers are small, fragrant, axillary or Cauliflorous, 5-spelled, imbricate, 5- petted and contorted. Stamens 10 united at the base, all perfect or 5 without anthers. Ovary 5- lobed, 5- celled without beak; styles 5 distinct, stigma capitates, ovules numerous in the cells. The present study is an attempt to fill the lacuna of knowledge regarding the phytochemical composition of the plant.

### MATERIALS AND METHODS

#### Plant material

Fresh bilimbi fruits were collected from Central Travancore region. All the collected plant material was identified using standard keys and floras. The collected plant parts were washed under running tap water followed by distilled water and dried and powdered.

### Preparation of extract

The powdered fruit samples were extracted by soxhlet extraction method, using three solvents -petroleum ether, chloroform and methanol. After extraction excess of the solvent was evaporated and the concentrated extracts were used for further analysis (Harborne, 1998).

### Physico-chemical analysis

The Physico-chemical studies include different standardization parameters like moisture content, total ash, acid insoluble ash and water soluble ash.

### Phytochemical studies

Preliminary phytochemical tests were performed for the detection of secondary metabolites such as alkaloids, flavonoids, coumarins, saponins and phenols (Harborne, 1998).

### Identification of Compounds by LC-MS

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

### Extraction

Powdered fruit samples were extracted with soxhlet apparatus for 3 hours using 50ml of methanol as solvent. The extract was then dried and dissolved in 10ml of methanol (HPLC grade, Merck) which was then filtered through 0.20mm membrane filter and use for analysis.

10µl of the filtered sample was injected to the manual injector using a micro syringe (1-20µl, Shimadzu). The mobile phase used was water:methanol (50:50) in an isocratic mode. The column used was Phenomenex RP 18. The separated compounds were then ionized using APCI methods (Atmospheric Pressure Ionization Method) and using the split mode (50:50). The class VP integrated software was used for the data analysis with Metwin 2.0 library.

### RESULTS

The phytochemical investigation of *A. bilimbi* fruits was carried out using standard protocols available. The results of Physico-chemical analysis of *Averrhoa bilimbi* fruit was given in Table 1. The total ash of the fruit was 5.54 %. The moisture content of the fruit was 93 ±1 %. The result of the phytochemical analysis was given in Table 2 and it

is clear that *A. bilimbi* fruit was enriched with different secondary metabolites especially flavonoids, Coumarins, Saponins and Phenols. Methanol extract was more diverse (Flavonoids, Coumarins, Saponin, Phenols) followed by chloroform (Flavonoids, Coumarins, Phenols) and petroleum ether extracts (Saponin). Results of LC-MS analysis chromatogram of major classes of metabolites in bilimbi fruit was given in Figure 1 and the integrated library search (METWIN 2.0) results with the list of compounds in Table No: 3.

## DISCUSSION

The present study has well demonstrated the phytochemical diversity of *A. bilimbi* fruit extract. The ash content was 5.54 % which is the measure of total mineral content of a material. Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter. Ash analysis is the basis of elemental analysis which is the part of proximate analysis for nutritional evaluation. The presence of flavonoids, Coumarins, Saponins and Phenols was well established in the present study which is also *at par* with available reports from different regions (Kumar *et al.*, 2013). The presence of primary metabolites like aldehyde, sugar and protein and secondary metabolites like cardiac glycoside, flavanoid, alkaloid, phenol, tannin and coumarin was reported from *A. bilimbi* leaves (Alisha and Raphael, 2016). Similar observations are reported by Yan *et al.* (2013) too. In short, *A. bilimbi* fruit can be recommended as a nutraceutical functional food.

The anti-inflammatory activity of flavanoids (Evans and Trease, 2002); anti apoptosis, anti aging, anti carcinogen, anti inflammation, anti atherosclerosis, cardiovascular protection, inhibition of angiogenesis activity of Phenolic compounds (Han *et al.*, 2007); antioxidant activity of Coumarins (Kostova *et al.*, 2011) and the membrane-permeabilising, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins (Francis *et al.*, 2002) are well established.

The LC-MS analysis of methanolic extract showed a highly complex profile containing approximately twenty components. It contains phytochemicals which may be useful for various herbal formulations as these compounds have anti-pyretic, analgesic, cardiac-tonic, anti-asthmatic and anti-inflammatory effects. Majority of the identified phytochemicals in the extract has proven biological effects. The neuroprotective effects of *trans* Resveratrol (Robb and Stuart, 2010); anti-oxidant effects of Umbelliferone (Singh *et al.*, 2010) are well documented.

*A. bilimbi* is medicinally important and is used as a folk remedy for many symptoms and showed significant pharmacological activities. The presence of immense phytochemicals in the plant can be utilized for production of novel drugs to combat various diseases.

## CONCLUSION

The traditional medicinal plant *A. bilimbi* was analyzed for its phytochemicals. The presence of flavonoids, Coumarins, Saponins and Phenols was observed. A total of 20 active principles were detected in the LC-MS investigation of the methanolic extract. Further studies are recommended to be undertaken to separate the exact compounds and to evaluate the mechanism of actions scientifically and synergistically.

**Table 1: Physico-chemical analysis of *Averrhoa bilimbi* fruit.**

Serial no.	Characteristic	Measurement
		% w/w Mean $\pm$ S.D
1	Total ash	5.54 $\pm$ 0.123
2	Acid insoluble ash	0.90 $\pm$ .051
3	Water soluble ash	3.01 $\pm$ 0.24
4	Moisture content	93 $\pm$ 1

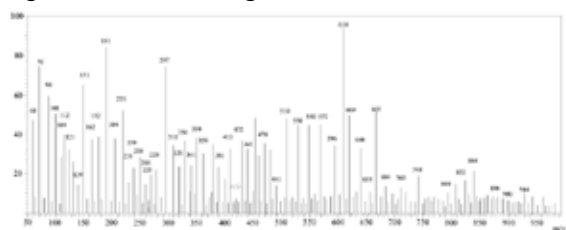
**Table no: 2. Qualitative chemical examination of various extracts obtained by soxhlet extraction of dried fruit powder of *A. bilimbi***

Sl. No	Test	Fruit extracts		
		Petroleum ether	Chloroform	Methanol
1	Alkaloids (Wagners Test)	-	-	-
2	Flavonoids	-	+	+
3	Coumarins	-	+	+
4	Saponin	+	-	+
5	Phenols	-	+	+

**Table 3: 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	Cinnamaldehyde	132.08
10	Benzyl cinnamate	238.29
11	Hydroxy-citric acid lactone	190.11
12	Benzyl alcohol	108.14
13	Phenethyl 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

**Fig 1: LC-MS Chromatogram of *A. bilimbi* fruits extract**



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