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Comparative Phytochemical screening tests of the ethanolic extracts of *Averrhoa carambola* and *Averrhoa bilimbi*

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Abstract: *Averrhoa carambola*, usually referred to as star fruit, is a versatile and enduring tropical plant that falls within the *Oxalidaceae* family. Similarly, *Averrhoa bilimbi*, also known as Bilimbi or Cucumber Tree, is a multipurpose tropical plant that belongs to the same family. Both of these plants possess significant economic worth due to the utilization of various plant components such as leaves, bark, flowers, fruits, seeds, roots, or the entire plant as alternative medicinal remedies for a range of ailments, particularly diabetes. The extracts derived from different components of *Averrhoa carambola* and *Averrhoa bilimbi* have been traditionally employed as traditional medicines for a wide range of ailments, demonstrating notable pharmacological properties. Therefore, it is imperative to conduct additional research in order to extract pharmacologically active molecules that can be utilized in the development of innovative pharmaceuticals for diverse medical conditions. This review aims to provide an overview and comparison of the photochemical constituents, conventional medicinal uses, and various biological activities, including anti-microbial, anti-inflammatory, cytotoxic, antioxidant, antifertility, and antibacterial activities, between *Averrhoa carambola and Averrhoa bilimbi*.

Key-words*:* *Averrhoa carambola*, *Averrhoa bilimbi*, Alkaloids, Flavonoids, Tannins, Steroids.

1. Introduction:

The development of humanity has occurred in conjunction with the utilization of indigenous herbs and therapeutic plants since ancient times. Humans have identified over 270,000 plant species, with the potential for the existence of nearly 400,000 species on Earth [1]. Humans have historically utilized indigenous plants for various purposes such as sustenance, energy, medicinal properties, construction materials, garments, and chemical synthesis. The herb has served as a significant medicinal resource since ancient times [2]. Given that more than 80% of the global population predominantly depends on herbal treatments for their essential health maintenance, it may be argued that the global healthcare system is primarily built on this traditional, nature-oriented medical approach [3]. Egyptian medical papyrus from the 14th century is the oldest documented record of the herb being used therapeutically. Traditional medicine has used plant materials in various forms such as decoctions, infusions, powders, and pastes to prevent and treat ailments and improve overall health [4]. In addition, humans have identified more than 270,000 plant species, however it is probable that up to 400,000 of them exist on Earth [5]. Insulin therapy has demonstrated efficacy in regulating blood glucose levels, although it does not effectively mitigate the associated complications, including retinopathy, nephropathy, and cardiovascular illnesses. The presence of significant complexity in individuals with diabetes is associated with a shorter life expectancy compared to those without diabetes [6].

1.1 The plant family: Oxalidaceae

The *Oxalidaceae*, also known as the wood sorrel family, consists of five genera of herbaceous plants, shrubs, and small trees. The genus Oxalis, which includes the bulk of the 570 species, is the most prominent. Individuals belonging to this particular family generally exhibit divided leaves, wherein the leaflets display "sleep movements" by expanding in the presence of light and contracting in the absence of light [7].
Habit: Predominantly perennial herbs, with infrequent occurrences of shrubs or trees (*Averrhoa*).
Stem: The stem of the plant consists of fleshy rhizomes or bulbous tubers that are either nearly aerial or have very short stems. It is composed of rosettes of radical leaves.

The leaf of *Biophytum sensitivum* is characterized by its alternating, pinnately or palmately complex structure. It is exstipulate and has long petioles. The leaflets are obcordate and fold at night, exhibiting noctinostic movements. In *Biophytum sensitivum*, the leaves are sometimes sensitive to touch. *Oxalis buplurifolia*, on the other hand, has leaves that are sometimes replaced by phyllodes. Cauline leaves have a rosettes arrangement (Biophytum) and are often found in a sessile state in several species of *oxalis* [8]. The inflorescence is either solitary or sub umbellate, with infrequent occurrences of racemose or cymose.

The floral characteristics of the plant include actinomorphism, bisexuality, and hypogyny. The calyx possesses five imbricate sepals and is positioned apically. The corolla is composed of five convoluted petals that are intertwined and exhibit either an apopetalous or basally sympetalous shape.
Capsules (berries), formed by the dehiscence of loculi, frequently with elastic properties. Seeds possess endosperm and often exhibit a basal aril. Pollination is done by insects.

The *Oxalidaceae* family exhibits a predominantly worldwide distribution. several regions including North America, Mexico, West Indies, Central America, South America, Eurasia, Africa, Atlantic Islands, Indian Ocean Islands, Pacific Islands, and Australia have been brought to Bermuda.

1.1.1 Photographs of Averrhoa carambola L and Averrhoa bilimbi

Figure 1.1: *Averrhoa carambola* leaves and *Averrhoa bilimbi* leaves

1.2 Medicinal value of Averrhoa carambola

Star fruits are widely recognized for their numerous advantages for health impacts. The aforementioned effects encompass antioxidant, hypoglycemic, hypotensive, hypocholesterolemia, anti-inflammatory, anti-infective, anti-cancer, and immune-enhancing properties. Star fruits are frequently employed in Ayurvedic and Traditional Chinese Medicine (TCM) for the treatment of various clinical conditions [9]. These conditions encompass fever, cough, diarrhea, chronic headache, inflammatory skin disorders (eczema), and fungal skin infections. In certain nations, mature fruit is employed for the treatment of hemorrhoidal hemorrhage.

1.3 Medicinal value of Averrhoa bilimbi

The fruit preserve is utilized as a therapeutic intervention for the management of coughs, beriberi, and biliousness. The fruit-derived syrup is utilized as a remedy for fever and inflammation, as well as for the purpose of halting rectal bleeding and alleviating internal hemorrhoids [10]. The leaves are administered in the form of a paste or poultice to alleviate itching, mumps swellings, rheumatism, and skin eruptions. These substances are administered to bites caused by toxic organisms [11]. The Malaysian population utilizes fresh or fermented leaves as a therapeutic intervention for sexual diseases. *Bilimbi* leaf infusion is a therapeutic treatment for coughs and is commonly consumed as a tonic after childbirth. According to reports, a floral infusion has demonstrated efficacy in treating coughs and thrush. The fruit of the *bilimbi* was utilized in folk

medicine to manage obesity in certain communities in India. Subsequent investigations were conducted to explore the antihyperlipidemic capabilities of the subject [12].

2. Method and Materials

2.1 Chemical investigation of the experimental plants

This study focuses on the investigation of the plant species of *A.carambola* and *A.bilimbi*.

Name of the plants	Family	Plant part
Averrhoa carambola	*Oxalidaceae*	leaves
Averrhoa bilimbi	*Oxalidaceae*	leaves

	Averrhoa carambola	*Averrhoa bilimbi*
Kingdom	*Plantae*	*Plantae*
Division	*Magnoliophyta*	*Tracheobionta*
Class	*Dicotyledonae*	*Magnoliopsida*
Sub-class	*Rosidae*	*Rosidae*
family	*Oxalidaceae*	*Oxalidaceae*
Genus	*Averrhoa*	*Averrhoa*
Species	*Carambola*	*Carambola*

Table 2.1: Taxonomic hierarchy of the investigated plants

2.1.1 Gathering and Classification of the botanical specimen

In July 2023, fresh leaves of *A. Carambola* and *A. Bilimbi* were obtained from the Banani officers' quarter in Dhaka, Bangladesh. According to Khandaker Kamrul Islam, a Senior Scientific Officer at the National Herbarium of Bangladesh, the leaves were recognized.

2.1.2 Plant extract preparation

A total of 2kg of *A. Carambola* leaves and a total of 2kg of *A. bilimbi* leaves were gathered and subjected to a thorough washing process using distilled water 2-3 times to eliminate any dust particles. Subsequently, the leaves were dried in a shaded area for a duration of 7-8 days. The desiccated botanical specimens were subsequently pulverized into finely powdered form utilizing a laboratory grinding mill. Individually 500 grammes of fine powder were achieved from each plant. The fine powders were immersed in solutions of 25% ethanol for a duration of 14 days. The extraction process employed in this study involved the maceration method. Subsequently, the resulting extract was subjected to filtration using Whatman No.1 filter paper and concentrated using a water bath at a temperature of 40°C. Subsequently, the desiccated extracts were refrigerated at a temperature of 4°C.

2.2 Phytochemical screening procedure

Sample	Test Solution	Observation	Inference
Test for Alkaloids: 2ml solution of the extract and 0.2 ml of dilute hydrochloric acid.	0.1 ml of Mayer's reagent.	Yellowish buff colored Precipitate was obtained	Presence of alkaloid
Test for Alkaloids: 2ml solution of the extract and 0.2 ml of dilute hydrochloric acid	1 ml of Dragendorff's reagent.	Orange brown precipitate was obtained	Presence of alkaloid
Test for Alkaloids: 2ml solution of the extract and 0.2 ml of diluted hydrochloric acid	0.1 ml of iodine solution (Wagner's reagent)	Reddish brown precipitate was obtained	Presence of alkaloid
Test for Alkaloids: 2ml solution of the extract and 0.2 ml of diluted hydrochloric acid	0.1 ml of picric acid solution (Hager's reagent)	Yellowish precipitate was obtained	Presence of alkaloid
Test for steroids:	1ml sulfuric acid	Chloroform layer acquired reddish brown	Presence of steroid

10 mg extract dissolved in 1ml chloroform		color and acid layer showed green fluorescence	

Table 2.2: Phytochemical screening of *A. Carambola* leaves and *A. bilimbi leave*

Test for flavonoids: 10 ml of solution extract hydrolyzed with 10% sulfuric acid. This was extracted with ether and divided into three portions.	a) 1ml diluted ammonia solution b) 1ml diluted sodium solution c) 1ml diluted sodium hydroxide solution	a) Greenish-yellow color was observed b) Pale yellow color was obtained c) Yellow color was obtained	a) Presence of flavonoids b) Presence of flavonoids c) Presence of flavonoids
Test for Reducing Sugar	5ml Fehling's A and B solution. Boiled for 5 minutes in a boiling water bath	Brick red color precipitate was not observed	Presence of reducing sugar
5ml of solution extract	2 drops of 5% α-naphthol solution (freshly prepared and added 1ml of sulfuric acid on the sides of the test tube)	Violet color ring was not formed at the junction of two lipids	Presence of reducing sugar

Table 2.3: Flavonoid screening test of *A. Carambola* leaves and *A. bilimbi leaves*

Test for Tannins	5ml solution of extracts	1ml of 10% potassium dichromate solution	Yellowish brown precipitate was not obtained	Presence of Tannins
Test for Glycosides	Small amount of extract	1ml of water and few drops of sodium hydroxide solution	A yellow color is formed	Presence of Glycosides
Test for Saponins	1ml solution of the extract	Diluted to 20ml with distilled water	Shaked for 15 minutes and formed 1cm foam layer	Presence of Saponins

Table 2.4: Phytocompounds screening of *A. Carambola* leaves and *A. bilimbi leaves*

Test For Carbohydrate	2ml of extract	2ml of conc. Sulfuric acid	A red or reddish violet ring is formed	Presence of Carbohydrate

Table 2.5: Carbohydrate screening test of *A. Carambola* leaves and *A. bilimbi leaves*

3. Result and Discussion

			A. Karambola		*A. Bilimbi*	
	Name of the test	**Test Solution**	**Present**	**Absent**	**Present**	**Absent**
1	Alkaloid Test	Mayer's Reagent	✓			
		Hagner's Reagent				
		Dragondorff's reagent	✓			
2	Flavonoid Test	Ammonia Solution	✓			
		Sodium Carbonate Solution			✓	
		Sodium Hydroxide Solution	✓		✓	
3	Reducing Sugar Test	Fehling A & B Solution	✓			
		Alpha- Naphthol Solution	✓			
4	Steroid Test				✓	
5	Saponin Test				✓	
6	Carbohydrate Test		✓		✓	
7	Glycoside Test		✓			
8	Tanin Test		✓		✓	

Table 3.1: Comparative Phytochemical Screening test results of *A. Carambola* and *A. Bilimbi*

The scientific foundation for the therapeutic use of A carambola's leaves and fruits in treating many diseases such as DM, hypertension, and microbiological infections has been established via extensive pharmacological research undertaken over the years. Unlike numerous pharmacological studies, there have been limited preliminary phytochemical examinations conducted on this plant. The substances that have been found thus far primarily consist of volatile oils, fatty acids, and long-chain hydrocarbons, which possess limited therapeutic properties.

On the other hand, the substances that have been found thus far in *A. Bilimbi* primarily consists of volatile oils, fatty acids, and long-chain hydrocarbons, which possess limited therapeutic properties. Although A. bilimbi has been extensively utilized in complementary medicine and has been scientifically validated for its pharmacological properties. There is a lack of comprehensive knowledge regarding the specific bioactive chemicals found within this botanical specimen. Due to the intriguing pharmacological characteristics exhibited by this plant, there exists a pressing necessity to ascertain and separate the bioactive components accountable for diverse biological actions. The biochemical mechanism of action of *A.bilimbi* can be better understood through the isolation and identification of bioactive chemicals found in various areas of the plant.

4. Conclusion

Understanding the bioactive components of the plant will serve as the foundation for creating a novel medication, either in its pure form or as standardized extracts, utilizing innovative and sophisticated technologies. Compounds that possess distinct pharmacological properties can potentially serve as promising candidates for future medication development. Therefore, the comparative phytochemical screening tests on *A. Carambola* and *A. Bilimbi* can have significant effects on the future isolation of potential bioactive compounds for the development of safe, and cost-effective drug treatment particularly for Diabetes Mellitus.

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