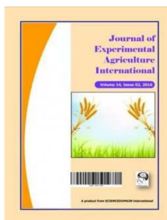

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Investigation of Phytochemical Components and Bioautography of *Garcinia mangostana* L. Methanol Leaf Extract

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of this work to identify chemical components of *Garcinia mangostana* leaves and test the antibacterial effect on *Pseudomonas syringe* pv. tomato and *Xanthomonas oryzae* pv. oryzae using bioautographic procedure. Phytochemical screening of methanolic extract showed that the leaf extract of *G. mangostana* rich in alkaloids, Flavonoids, Saponins, Tannins, Phenol, Terpenoids, Anthraquinone and Cardiac glycosides. A simple bioautographic procedure, involving spraying suspensions of the bacteria on thin layer chromatography (TLC) plates developed in solvents of varying polarities was used to detect the number of antibacterial compounds present in the extract. This activity was indicated by white spots against a red background on the TLC plates after spraying with 5% TTC aqueous solution. *P. syringae and X. oryzae* were inhibited by the most compounds separated on the TLC plates from the extract.

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Keywords: TLC; antibacterial; Xanthomonas oryzae; Pseudomonas syringe; phytochemical screening.

1. INTRODUCTION

Since centuries, different kinds of herbs and plants found naturally have been used as the key ingredients in traditional remedies to treat various ailments; to this day, these are extensively used worldwide [1]. Phytochemicals that occur naturally in plants provide them with a characteristic protection mechanism. They are responsible for the colour, smell, and taste found in fruits and vegetables. They are also used in the production of medicines and agrochemicals. The antimicrobial characteristics of plants are probably connected to their capability to produce chemical compounds of fairly complicated structures with antimicrobial action, including flavonoids, alkaloids, taninins, isoflavaoids, saponin, cumarin, terpenoids, glycosides, phenol, and organic acids [2,3].

The secondary metabolites may be obtained with the help of various solvents and the active metabolites and their structures can be clearly understood through different techniques [4]. An important procedure used to evaluate phytochemical compounds is thin-layer chromatography (TLC). With the help of different low-cost TLC procedures, which reveal many findings on the same TLC plate within a short period of time, phytochemicals in crude plant extracts can be isolated [5]. According to Sajewicz et al. [6], the TLC process of using silica gel is a critical supplementary fingerprint technique to detect phenolic acids and flavonoids fractions from diverse species of sage.

The laboratory procedure to identify elements that impact the growth rates of test organisms in complex compounds is called bioautography. This is just one of the many investigation techniques used to check for antimicrobial movement [7]. In bioautography, plant extracts and their uncontaminated phytochemical compounds are assessed alongside human pathogenic and plant pathogenic microbes [8]. One more antimicrobial procedure that provides valuable data of the position of active spots on TLC that constrain tested microorganisms is direct bioautography. This active position was identified and displayed with the calculation of retention factor (Rf) values [9]. A good division on TLC with good solvents will yield many bands and these active bands obtained can be tested against tested microoganisms [10]. The antimicrobil or inhibition spots will be characterised by clear white against purple red background [11,8].

Earlier studies indicated that mangosteen leaf extracts from its different parts comprises many secondary metabolites such as triterpenoids, flavonoids, benzophenones, prenylated, and oxygenated xanthones [12]. Earlier investigations of mangosteen have shown the occurrence of flavonoids, phenols, and triterpenoids [13,14]. Hence, the present study aims were to determine the existence of phytochemicals compounds in mangosteen methanol leaf extract and limit the active antibacterial compounds from mangosteen methanol leaf extract on thin layar chromatography using direct bioautography.

2. MATERIALS AND METHODS

2.1 Preparation of Mangosteen Leaf Extract

The Mangosteen leaves powder was immersed in 250 mL of methanol and shaken with a shaker for 48 hours. The macerate was filtered with Whatman no. 1 filter papers (ALBERT^R) after 2 days of immersion. Finally, the extract was purified and vaporised utilising a rotary evaporator and stored at 4℃ for further use [15].

2.2 Primary Phytochemical Screening

2.2.1 Alkaloids

For the Alkaloids screening, 200 mg of plant extract was dissolved in 10ml methanol followed by filtration using gauze. 1 ml of the filtrates was then mixed with 2-3 drops of Wagner's reagent (dissolved 2 g of iodine and 6 g of KI in 100 mL of water). Creamish, brownish red or orange precipitate indicated the presence of Alkaloids [16].

2.2.2 Anthraquinones

For the Anthraquinones screening, a mixture of 0.5 g of the extract and then 1 mL of H_2SO_4 was heated and filtered while it was still hot about 5 mL of chloroform was added to this filtrate and mixed well. The chloroform layer was transferred into another test tube with a pipette and 1 mL of dilute ammonia was added to it. The solution thus obtained was checked for any change in colour.

2.2.3 Terpenoids (Salkowski test)

A mixture of 0.5 g of the extract and 2 mL of chloroform was prepared and 3 mL of concentrated H_2S0_4 was then slowly added to form a layer. A reddish brown coloration of the interphase shows the existence of terpenoids.

2.2.4 Shinoda's test for flavonoids

Approximately 500 mg of the extract was added to 5 mL of ethanol and was slightly heated and then filtered. Few pieces of magnesium chips were added to the filtrate along with a few drops of concentrated HCl. An orange, pink, or red to purple coloration was considered as validation for the occurrence of flavonoids [1].

2.2.5 Saponins

A mixture of 1 g of powdered extract dissolved in 10 mL of distilled water was boiled and then filtered. The filtrate was then mixed with 3 ml of distilled water with stirring for about 5 minute. Formation of foam after shaking was considered as validation for the presence of saponins [17].

2.2.6 Tannins

A mixture of 500 mg of extract with 10 mL of distilled water was filtered and then a few drops of 1% ferric chloride solution were added to it. Occurrence of a blue-black, green, or blue-green precipitate shows the existence of tannins.

2.2.7 Cardiac glycosides (Keller-Killiani test)

About 0.5 g of extract dissolved in 5 mL of water was mixed with 2 mL of glacial acetic acid and one drop of ferric chloride solution. This mixture was then added to 1 mL of concentrated sulphuric acid. A brown ring at the interphase shows the occurrence of a deoxy sugar typical of cardenolides. A violet ring may be present under the brown ring, whereas a greenish ring may appear in the acetic acid layer just above the brown ring and slowly spread all through this layer.

2.2.8 Phenol

A mixture of a few drops of ferric chloride solution and 2 mL of the test solution was prepared. Bluish green or red colour shows the existence of phenol [18].

2.3 Thin Layer Chromatography (TLC) Analysis

For the sake of standardisation in the testing, identical TLC plates measuring 2 x 5 cm were obtained by cutting 60 F_{254} (Marck) silica gels of size 20 x 20 cm into small bits. Every small plate was measured and denoted with 0.5 cm at the bottom and the top with a pencil and ruler, that left a loading space of 4 cm. Various solvent systems with diverse polarities were made and TLC evaluation was performed to choose the solvent that displays the best resolution. The methanol leaf extract sample was smeared on pre-coated TLC plates with the help of capillary tubes and developed in a TLC chamber with appropriate mobile phase. These developed TLC plates were air dried and viewed under ultra violet light UV at 254 nm and 366 nm. The retention factor (Rf) values which indicate the activity of the analysis were considered for different samples. The below formula was used to compute Rf value:

Retention factor (Rf) = (Distance travel by solute / Distance travel by solvent)

All the TLC plates were observed right after drying using UV at 254 nm and 366nm in a UVTLC viewer. The Rf value of the different pots which were viewed was measured [19].

2.4 Bioautography

About 10 mg/mL of methanol extract was added onto the TLC plates in a narrow band and eluted with two mobile solvent systems – hexane and acetone. The developed plates were then dried under a stream of fast moving air for two days to get rid of any traces of solvent on the plates. Overnight cultures of bacterial tests developed on MH broth and bacterial organism with a density of approximately 2×10^6 cfu/mL were utilised for *P. syringae* pv. *tomato* and *X. oryzae* pv. *oryze*. The procedure of thoroughly spraying the prepared chromatograms with the bacterial suspension was done in a Laminar flow cabinet. The TLC plates were then cautiously put into sterilised petri dishes packed with wet tissues to keep the moisture within and incubated for 24 hours at 30ºC. On the completion of 24 hrs, the TLC plates immunised with the bacterial suspension were exposed to 5% TTC aqueous solution and kept in a laminar flow cabinet for 3 hrs so that TTC could mark the TLC plate background with sustainable bacteria cell into red, and the background of the inhibition zones

would remain white. The inhibition zones denoted by certain Rf values were measured and selected as the active compound capable of preventing bacteria growth [11].

3. RESULTS

3.1 Primary Phytochemical Screening

The qualitative approximation of phytochemical components in the mangosteen methanol leaf extract had shown the existence of anthraquinones, carbohydrates, terpenoids, saponins, flavonoids, glycosides, tannins, and phenol as presented in Table 1.

The active constituents of the raw material, the nature and polarity of extraction solvent, and separation procedure are the features that confirmed the activity of natural plant extracts [20]. In the extraction of bioactive compounds, the key factor to be considered is solvent polarity – the solubility of phenolic compounds [21] is better if the polarity is higher. Polar compounds that have a high degree of solubility in high polarity solvent are called phenolic compounds [22]. To extract phenolic from fresh products, the regularly used solvent is methanol [23,24]. Phenolic compounds are one of the main families of secondary metabolites in plants. A large number of the active compounds are situated in the vacuoles inside plant cells, in the polar soluble division which is removed quite effortlessly using polar solvents such as methanol [25].

Flavonoids are ubiquitous in photosynthesising cells and therefore occur widely in the plant kingdom. Several previous studies performed had proved flavonoids possess the ability of being antimicrobial agents against pathogenic microbe [26]. Terpenoids are a large family of natural products belong to the group of saponin exhibiting a wide range of biological activities such as antibiotics, anti-inflammatory, anti-HIV and anti-tumor effects; hypotensive agents; sweeteners; insecticides; anti-feedants; phytotoxic agents; perfumery intermediates; and plant growth hormones [27].

3.2 TLC Analysis of Methanol Crude Extract

In this evaluation, the methanol crude extract of mangosteen leaf was portioned and divided with TLC with a mixture of acetone and hexane in the ratio of 4:6 (v/v) as solvent system. This produced a reasonable separation of methanol crude extract with visible bands in normal light without any visualisation aid as represented in Fig. 1(A). The effectively developed TLC plates were also observed with short and long UV light (254 and 365 nm) respectively and the divisions attained are represented in Fig. 1(A) and (B), respectively.

The leaves of *G. mangostana* were reported to comprise polar compounds established by the extract yields [28]. Earlier research showed that acetone and *n*-hexane are the good mobile phases for TLC investigation [29,30].

Table 2 illustrates that when various visualisation techniques are used, different ranges of R_f value are obtained. The R_f value for TLC visualisation under normal light fell in the range of 0.16 and 0.93. Whlile under short UV wavelength (254 nm), the R_f value fell in the range of 0.26 and 0.93 and under long UV wavelength (365 nm), the R_f value fell in the range of 0.26 and 0.86.

Table 1. Phytochemical screening of mangosteen methanol leaf extract

Chemical constituent	Positive results	Methanol leaf extract
Alkaloids	Creamish, preciptate	+
Anthraquinone	Yellowish	+
Terpenoids	Reddish brown	+
Flavonoids	Orange	+
Saponins	Small bubbles	+
Tannins	Blue-green	+
Phenol	Bluish green	+
Cardiac glycosides	Brown ring	+

+ = *Detected*

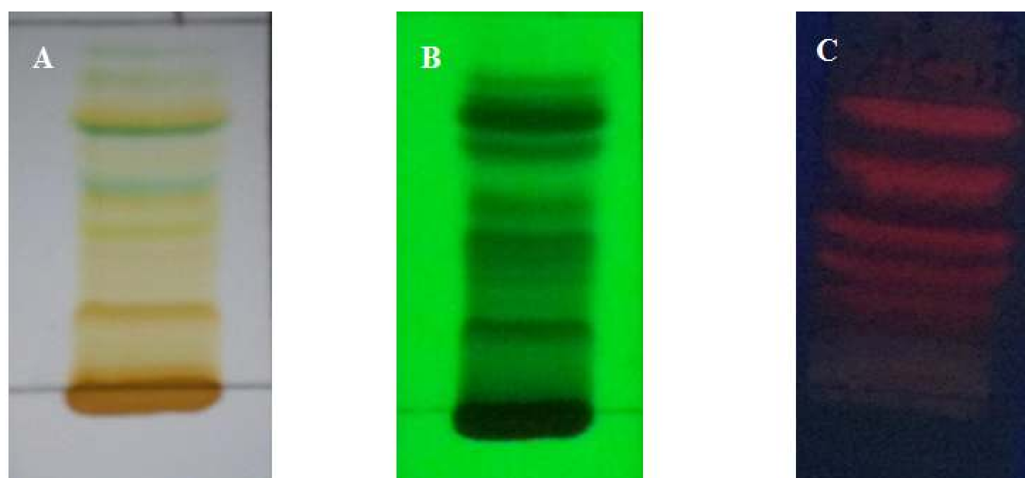

Fig. 1. TLC spot visualization under daylight (normal light) (A), short UV wavelength (245 nm) (B), and long UV wavelength (365 nm) (C)

Table 2. The retention factor (Rf) for *G. mangostana* L. methanol leaf extract with different visualization techniques

No. of band	Rf		
	Normal light	Short UV wavelength (254 nm)	Long UV wavelength (365 nm)
1	0.93	0.93	0.86
2	0.86	0.86	0.83
3	0.73	0.83	0.76
4	0.66	0.76	0.73
5	0. 53	0.73	0.63
6	0.46	0.66	0.53
7	0.43	0.56	0.33
8	0.36	0.53	0.26
9	0.33	0.46	
10	0.23	0.43	
11	0.16	0.26	

In this research, acetone and hexane in the ratio of 4:6 respectively was a great mobile phase as it allowed to distinguish each band which characterised different phytochemicals effectively. This mobile phase revealed ten bands under short UV wavelength, eight bands under long UV wavelength and eleven bands under normal light with different R_f values while some were similar distance.

3.3 Direct TLC Bioautography Assay

In this testing, a good separation brought about with a proper solvent system of TLC presented many inhibition zone spots with various retention factor (R_f) values on chromatograms as represented in Fig. 2. The testing was carried out for *P. syringae* pv. *tomato* and *X. oryzae* pv. *oryzae*.

The inhibition zone spot range denoted by R_f values detected for antibacterial activity in contrast to *P. syringae* was at 0.33, 0.46, 0.66, and 0.86. The inhibition zone spot noted only for *P. syringae* pv. *tomato* under the R_f values at 0.33 and 0.66 while R_f values at 0.16, 0.36, and 0.93 were detected only for *X. oryzae* pv. *oryzae.* From the test, practically both the R_f values recorded at 0.46 and 0.86 were shown as inhibition zone spots for both of the plant pathogenic bacteria tested.

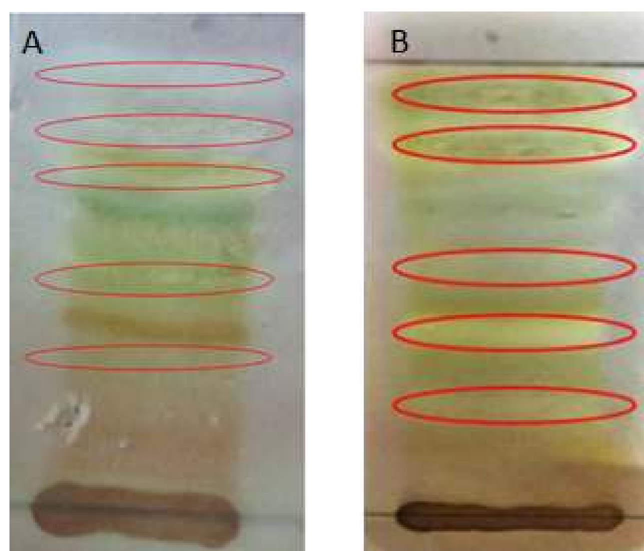

Fig. 2. Direct bioautography of *G. mangostana* L. methanol leaf extract against *P. syringae* (A), and *X. oryzae* (B). Inhibition zones were circled due to low light quality during the photography session

4. CONCLUSION

In this current investigation, the mangosteen leaves comprise possibly antibacterial constituents that are beneficial for the growth of natural bactericide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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