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Research Article

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HPTLC profile of quercetin in three cultivars of *Allium sativum* and its antimicrobial activity against bacterial cultures

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ABSTRACT

This study was subjected to analyse the technique for the determination of quercetin, a flavonoid in garlic (Allium sativum L.) from three different cultivars of bulb onion (G1, G2 and G3) which were extracted with petroleum ether, chloroform, methanol and water. Also this study was aimed to determine the antimicrobial activity of four solvent extracts of garlic cultivar against ten MTCC bacterial pathogens. Quercetin in garlic was determined by analytical technique, HPTLC and the antimicrobial activity was performed by well diffusion method. Quercetin was determined in all the three cultivars with Rf value 0.68. Quercetin was also extracted in all the four solvent extracts of garlic cultivars. Apart from quercetin, few more compounds also isolated. More compounds were isolated in G1. In the determination of antimicrobial activity of onion extracts against MTCC cultures revealed that onion cultivar was highly active against Staphylococcus aureus, it showed more than 25mm zone of inhibition. This study showed that onion has good broad spectrum bioactivity against microorganisms revealed that it contains phytochemicals which can be used as neutraceuticals.

Keywords: *Allium sativum*, HPTLC, antimicrobial activity, bacterial pathogens, zone of inhibition, quercetin

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INTRODUCTION

Garlic (*Allium sativum*) has been utilized all the way through the past, serving as food supplement as well as treatment for many diseases. The father of Ayurvedic medicine, Charak stated that garlic helped to uphold fluidity of blood and strengthened the heart [1]. Many different benefits of garlic on human health, including anticarcinogenic, antifungal and antibacterial properties has revealed in recent researches [2]. It is these benefits that have driven the extensive research on the phytochemical components of garlic to determine the component responsible for health benefits. Most of the phytochemicals in garlic have been directly linked to haying an inhibitory effect on chemical carcinogenesis [3].

One of the major phytochemicals are flavonoids, a phenolic substance found in wide range of higher plants and so far more than 8150 flavonoids have been reported [4]. Biological activities of flavonoids such as antiallergenic, antiviral, anti-inflammatory and vasodilating effect were suggested in many of the researches [5]. Quercetin is a flavonoid occurs in food plants including apple, berries, Allium genus, tea and vegetables [6]. Due to numerous health benefits of such phytochemicals, recently great attention is being given to estimate the quality, efficiency and standards of the herbal medicines [7]. Quercetin is the important bioactive component of *Allium* species; therefore there is a urgent need to develop a rapid and simple method for the determination of the phytochemical compounds using instruments like HPTLC. In aim of this present study is to determine quercetin in garlic cultivars collected

from three different cultivation sites by HPTLC method and to determine its antimicrobial activity against bacterial cultures.

EXPERIMENTAL SECTION

Collection of plant materials

Allium sativum from different cultivation site such as Poomparai, Kodaikanal (G1), Vagudapatti, Theni (G2) and Pannaikadu, Kodaikanal (G3) were collected and brought to the laboratory for further analysis.

Processing of plant materials

The collected *A.sativum* bulb from different cultivation sites were cleaned thoroughly and dried under shade. The dried bulb was blended into fine powder and stored in air tight container at room temperature.

Preparation of extracts

The organic solvents such as petroleum ether, chloroform, methanol and distilled water was used for the extracting the bioactive compounds from bulb of *A.sativum*. The extraction was done using soxhlet apparatus. The extract dried using vacuum evaporator and stored in air tight containers.

HPTLC Fingerprinting Profile

Sample Preparation

A HPTLC analysis was performed using CAMAG LINOMAT V (Switzerland) for the various solvent extracts dissolved with respective HPTLC grade at the rate of 1 mg/ml.

Sample Application

The samples (6µl) were loaded on to the HPTLC plate at 8mm bands length in the 10×10 silica gel 60 F_{254} TLC plate along with nitrogen gas supply for simultaneous drying of bands, by means of a CAMAG (Switzerland) Linomat V sample applicator fitted with a 100µl syringe (Hamilton Bonaduz, Switzerland). The sample loaded plate was kept in TLC twin trough developing chamber with respective mobile phase and the chromatogram was developed to a distance of 70mm. Densitometric scanning was performed with a CAMAG TLC scanner 3 in reflectance absorbance mode at 254 nm, under control of CAMAG WINCATS Planar Chromatography Manager Software (version 1.4.2). HPTLC fingerprinting and chromatograms were developed and recorded.

Determination of antimicrobial activity

The Muller Hinton agar (MHA) plates were swabbed with bacterial pathogens and well of 8mm diameter was punched into the MHA medium and filled with 10-50µl (100-500µg) of solvent extract. The plates were incubated at 37˚C for 24 hours. After incubation period, the diameters of zone of inhibition produced by the extract with different bacterial pathogens in different plates were measured and recorded.

RESULTS

HPTLC finger printing profile

The bands in the solvent extract of garlic cultivars found matched with standard quercetin with Rf value 0.68. 5 more compounds were separated in petroleum ether extract of G2 and 2 additional compounds were separated in G1 and G3 (Figure 1). These two bands coincide with each other indicating that similar compound was separated. 5 more compounds were separated only in methanol extract of G1 in which one compound matched with a compound in G2. G3 also found with 3 compounds additionally in the methanolic extract.

An additional compound with Rf value 0.92 was separated in chloroform extract of all the three garlic cultivar in which the band in G1 and G2 found matched. Water extract of G1 was identified with 3 more bioactive compounds in which 2 compounds were found matched with G2 and G3 with Rf values 0.89 and 0.9. In water extract of garlic, all the three cultivars showed good separation of bioactive compounds. In HPTLC analysis, more compounds were separated in methanol and water extract of G1 and petroleum ether extract of G2 (Figure 1). In this study, more compounds were isolated in G1, so G1 was considered as superior cultivar and used in further antimicrobial studies.

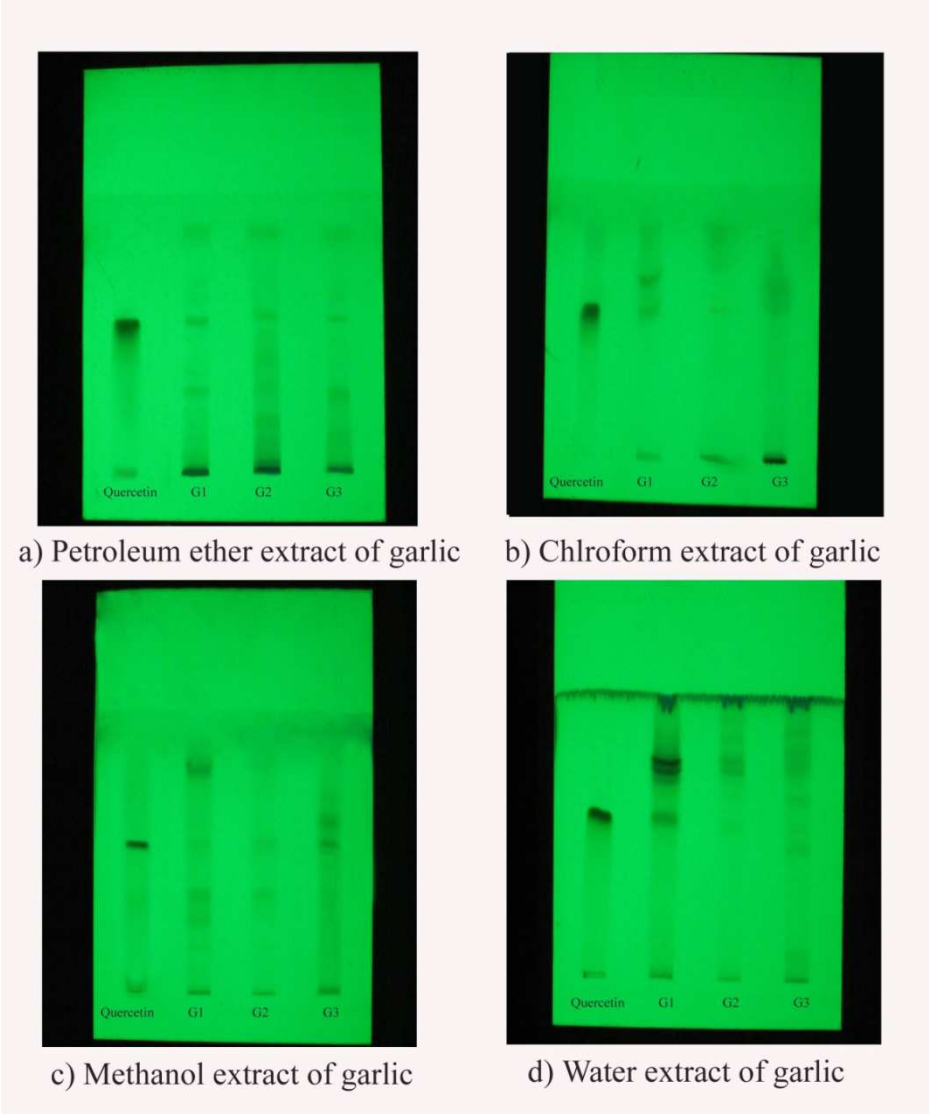

Figure 1. HPTLC profile of garlic cultivars

Antimicrobial activity of garlic cultivar against bacterial cultures

The garlic cultivar which showed more phytochemicals in the HPTLC analysis was tested for its antimicrobial activity against bacterial cultures. The solvent extracts of garlic exhibited good antibacterial activity against all the ten MTCC cultures tested. Petroleum ether, methanol, water and chloroform extract showed maximum activity against *Staphylococcus aureus* and the antibacterial activity determined was 24.5±0.4mm, 24.3±0.2mm zone, 23±0.5mm zone and 18.5±0.4mm zone of inhibition respectively in 500 µl concentrations (table 1).

Methanol and water extract of garlic showed maximum activity against *Pseudomonas aeruginosa* in the range of 21.2±0.2mm zone of inhibition and 18.3±0.2mm zone of inhibition in 500 µl concentrations respectively. *Klebsiella pneumonia* showed maximum sensitivity (15.5±0.5mm zone of inhibition in 500 µl concentrations) to methanol extract and exhibited no sensitivity to the least concentration of petroleum ether, chloroform and water extract and showed less activity towards the highest concentration of chloroform extract of garlic (10.8±0.6mm zone). Water and petroleum ether extract exhibited 14.1±0.2mm and 12.8±0.2mm zone in 500 µl concentrations respectively. Methanol and petroleum ether extract exhibited 17.8±0.2mm zone and 16.1±0.2mm zone of inhibition against *E.coli* in 500 µl concentrations followed by water and chloroform extract of garlic (Table 1).

Clinical Pathogens	Zone of Inhibition (mm)/Concentration of extract (µg)																			
	100				200				300				400				500			
	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water	Petroleum ether	Chloroform	Methanol	Water
K.pneumonieae	0	0	9.1 ±0.2	0	0	0	9.3 ±0.2	0	0	0	11.1 ±0.2	9.1 ±0.2	8.8 ±0.2	8.6 ±0.2	13 ±0.5	11.3 ±0.2	10 ±0.4	9.3 ±0.4	13.8 ±0.7	12.3 ±0.5
E.coli	0	0	9.8 ±0.2	8.8 ±0.2	8.8 ±0.2	0	12.1 ±0.2	10 ±0.5	9.1 ±0.2	8.8 ±0.2	14.3 ±0.2	12.1 ±0.2	11.5 ±0.4	10.3 ±0.4	15.5 ±0.5	13.1 ±0.2	13.8 ±0.2	11 ±0.7	16.3 ±0.2	13.3 ±0.2
S.pyogenes	0	9.8 ±0.2	9.1 ±0.2	10	8.8 ±0.2	11 ±0.4	9.5 ±0.5	12.1 ±0.2	9.8 ±0.2	12.8 ±0.2	11.3 ±0.2	12.3 ±0.2	10.8 ±0.2	13.3 ±0.2	12.1 ±0.2	13.3 ±0.5	13 ±0.4	14.1 ±0.2	12.5 ±0.5	15.3 ±0.2
P.auroginosa	0	0	14.8 ±0.2	12.1 ±0.2	0	0	17.1 ±0.2	13.8 ±0.2	8.8 ±0.2	0	19.1 ±0.2	14.6 ±0.2	11.5 ±0.4	8.6 ±0.2	20.3 ±0.2	14.8 ±0.2	11.8 ±0.2	9.6 ±0.6	20.5 ±0.5	15.3 ±0.2
E.aerogenes	9 ±0.4	0	13.1 ±0.2	10.1 ±0.2	11.5 ±0.4	8.8 ±0.2	14.3 ±0.2	13.1 ±0.2	13.5 ±0.4	9.1 ±0.2	16.5 ±0.5	13.3 ±0.2	15.3 ±0.4	9.1 ±0.2	17.3 ±0.5	15 ±0.5	18 ±0.4	10.1 ±0.2	19 ±0.5	15.3 ±0.2
S.aureus	12.3 ±0.2	9.3 ±0.4	10.8 ±0.2	15.3 ±0.2	13.8 ±0.6	12 ±0.4	14 ±0.5	17.1 ±0.2	14.6 ±0.4	12.6 ±0.4	16.1 ±0.2	17.3 ±0.2	18.5 ±0.4	14.3 ±0.4	19.3 ±0.2	18.5 ±0.5	21.17 ±0.2	16.5 ±0.4	21.5 ±0.5	21.3 ±0.2
P.vulgaris	0	10	16.8 ±0.2	11.5 ±0.5	8.6 ±0.2	10	18.3 ±0.2	12.3 ±0.2	9.1 ±0.2	11 ±0.7	18.5 ±0.5	13.3 ±0.5	10.8 ±0.6	13.3 ±0.2	20.8 ±0.2	13.8 ±0.2	12.5 ±0.4	13.3 ±0.2	21.3 ±0.2	14.3 ±0.2
S.typhi	8.8 ±0.2	0	10.3 ±0.5	0	10.1 ±0.2	0	12.8 ±0.2	0	10.8 ±0.2	9	14.1 ±0.2	9.1 ±0.2	12.1 ±0.2	9.3 ±0.4	14.3 ±0.5	10.3 ±0.2	13.8 ±0.2	11.5 ±0.5	15.3 ±0.2	11.8 ±0.2
B.subtilis	0	0	0	0	0	10.5 ±0.5	0	0	0	11	9.1 ±0.2	0	9.8 ±0.2	12 ±0.4	9.3 ±0.2	10.1 ±0.2	10.5 ±0.4	12.6 ±0.4	11.3 ±0.2	10.3 ±0.2
A.hydrophila	0	9	9.1 ±0.2	0	8.8 ±0.2	10	10.1 ±0.2	9.1 ±0.2	9.1 ±0.2	12.8 ±0.2	10.5 ±0.5	9.3 ±0.2	11 ±0.4	13 ±0	13.17 ±0.2	10.1 ±0.2	12.1 ±0.2	15.5 ±0.5	13.5 ±0.5	10.3 ±0.2

Table 1. Antimicrobial activity of solvent extracts of garlic against MTCC cultures

Streptococcus pyogenes was sensitive to water, chloroform, petroleum ether and methanol extract of garlic in the range of 18.3±0.2mm zone of inhibition for water extract, 16.1±0.2mm zone for chloroform extract, 15.3±0.4mm for petroleum ether extract and 14.5±0.5mm zone of inhibition for methanol extract in 500 µl concentrations. Methanol extract of garlic showed maximum antibacterial activity against *Enterobacter aerogenes* in the range of 14±0.5mm to 21.3±0.5mm in 100 to 500 µl concentrations. *Proteus vulgaris* was resistant to the least concentrations of petroleum ether of garlic.

Methanol and water extract showed maximum activity against *Salmonella typhi* in the range of 17.3±0.5mm zone of inhibition and 14.3±0.2mm zone respectively in 100, 200, 300, 400 and 500 µl concentrations. *Salmonella typhi* showed resistant to least concentration of chloroform extract of garlic. *Bacillus subtilis* was sensitive to methanol extract of garlic in the range between 9.1±0.2mm and 13.3±0.2mm zone of inhibition in 100 and 500 µl concentrations. Chloroform, water and petroleum ether extract does not showed antibacterial against *Bacillus subtilis* in the least concentrations. Chloroform extract of garlic showed maximum activity (15.3±0.2mm zone in highest concentration) against *Aeromonas hydrophila.* The maximum activity exhibited by water extract was 11.3±0.2mm zone of inhibition in 500 µl concentrations (table 1) and showed less activity for water extract in higher concentration. *Aeromonas hydrophila* showed resistant to petroleum ether and water extract in the least concentration.

DISCUSSION

The results showed linearity and correlation coefficient within the range of concentration. There are good correlation between peak area and the corresponding concentration of quercetin. Stationary phase silica gel TLC plate and mobile phase chloroform: methanol (8:2) had given good separation of quercetin. The identification of band of quercetin in the bean extract using HPTLC was confirmed by Mishra *et al.* [8]. The linear calibration curves of quercetin were found to be linear over the range of 100-200 ng in their study.

Seven distinct spots including those at the origin and at the solvent front, numbered consequently from the base by TLC were obtained in onion by Bandyopadhyay *et al.* [9]. In their study, the highly polar compounds do not migrate with the solvent systems used (petroleum ether: diethyl ether, 8:2) since the separation was based on the polarity of the compounds present. Itakura *et al*. [10] separated one specific sapogenin in garlic, β-chlorogenin, by TLC. In Parmar *et al*. [11] study, the quercetin was well separated in mobile phase toluene: ethyl acetate: acetone: formic acid (5:2.5:7.5:0.5). In this study, quercetin was well separated by the solvent system chloroform: methanol (8:2).

The study of Daka [12] revealed that antibacterial activity of the fresh *Allium* extract showed greater effectiveness against tested organisms. He studied on the antibacterial activity of garlic on *S.aureus.* The dilute solutions of garlic can completely inhibit the growth of *S.aureus* at the concentration of more than 7.5mg/ml. According to Onyeagba and his colleagues [13] the crude extract of garlic did not exhibit any invitro inhibition on the growth of test organisms including *Staphylococcus* sp. Various bacterial strains resistant to antibiotics such as methicillin resistant *Staphylococcus aureus* as well as other multidrug resistant organisms were all found to be sensitive to allicin, a major bioactive compound in garlic [14].

The maximum inhibition zone for garlic extract was observed against *Enterbacter* sp. (40mm) and minimum zone was observed against *S.aureus* (25mm). Among the gram negative organisms, the maximum zone was observed against *S.typhi* (50mm) and minimum zone against *Proteus* sp. (20mm) in Hindi [15] study. *E.aerogenes* was not susceptible to aqueous extract of garlic while *S.typhi* was susceptible (22±0.4mm, 24±0.6mm and 26±0.4mm in 300, 400 and 500 µg concentration) in Shobana et al [16] study. Also they reported that alcoholic extract of *A.sativum* was highly effective against all the bacterial species that was taken for the study. Al-Delaimy and Ali [17] reported that 4% (w/v) fresh garlic extract inhibited the growth of *S.aureus, E.coli* and *S.typhi.*

In Karuppiah and Rajaram [18] investigation, the garlic cloves extracts exhibited high degree of inhibitory activity against most of the seven tested organisms. Among the clinical pathogens, *P.aeruginosa, E.coli, Bacillus* sp., *S.aureus* and *Enterobacter* sp. were the least inhibited by garlic extracts. The diameter of zone of growth inhibition varied between 7mm and 19mm in garlic. The garlic cloves alcoholic extract showed highest diameter of zone of inhibition of 19.45mm against *P.aeruginosa* followed by *E.coli* (18.50mm) and *Bacillus* sp. (16.5mm). It showed similar zone of inhibition of 13.50mm in diameter against *Proteus* sp., *Enterobacter* sp. and *S.aureus.*

In wan *et al*. [19] study, garlic was observed to have an antimicrobial effect on *E.coli*. All the test organisms were inhibited by aqueous garlic extract up to 25% concentration and the activity was a linear function of concentration. At 100%, the maximum zone of inhibition was observed against *B.subtilis* (54mm) a gram positive organism and the

minimum was observed against *Proteus* sp. (22mm), a gram negative organism in Durairaj *et al*. [20] study. This indicated that aqueous garlic extract has the potential of a broad spectrum of activity against both gram positive and gram negative bacteria. De *et al*. [21] observed the antimicrobial activity of garlic invitro against *B.subtilis* and *E.coli*. Arora and Kaur [22] observed a significant bactericidal effect of garlic extract against *Staphylococcus* sp. and *S.typhi*.

CONCLUSION

HPTLC analysis of solvent extracts of garlic revealed the presence of quercetin in all the three garlic cultivars. In this the G1 showed high number of phytochemicals, hence that particular cultivar is subjected for antimicrobial activity against ten MTCC bacterial cultures. All the four solvent extracts of *Allium* extract completely inhibits the growth of the microorganisms used in this study. This may due to the extract preparations, different concentrations of the active compounds present in the extract and their interactions in the culture media. Antibiotics were used for therapy, but many of the pathogenic bacteria are resistant. Natural products of higher plants may offer a new source of antibacterial agents and from this result it is clear that the medicinal value of *Allium* sp. is comparable to the present day antibiotics.

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