ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 3, 2015

ISSN - 0974-2441

Research Article

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY PROFILE OF QUERCETIN IN THREE CULTIVARS OF *ALLIUM CEPA* AND ITS ANTIMICROBIAL ACTIVITY AGAINST BACTERIAL CULTURES

PACKIA LEKSHMI NCJ[1]*, VIVEKA S[2], VISWANATHAN MB[3], MINI SHOBI T[3], JEEVA S[4], RAJA BRINDHA J[4]

[1]Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India. [2]Department of Biotechnology, Udaya School of Engineering, Vellamodi, Kanyakumari, Tamil Nadu, India. [3]Department of Plant Science, Bharathidhasan University, Trichy, Tamil Nadu, India. [4]Department of Microbiology, Udaya College of Arts and Science, Vellamodi, Kanyakumari, Tamil Nadu, India.
Email: packia_3779@yahoo.co.in

Received:27 February 2015, Revised and Accepted: 23 March 2015

ABSTRACT

Objective: This study was subjected to analyze the technique for the determination of quercetin, a flavonoid in onion (*Allium cepa* L.) from three different cultivars of bulb onion (O1, O2, and O3) which were extracted with petroleum ether, chloroform, methanol, and water. Furthermore, this study was aimed to determine the antimicrobial activity of four solvent extracts of three onion cultivars against 10 microbial type culture collection (MTCC) bacterial pathogens.

Methods: Quercetin in onion was determined by analytical technique, high-performance thin layer chromatography, and the antimicrobial activity were performed by the well diffusion method.

Result: Quercetin was determined in all the three cultivars with retention factor value 0.68. Quercetin was also extracted in all the four solvent extracts of onion cultivars. Apart from quercetin, few more compounds also isolated. More compounds were isolated in O3. In the determination of the antimicrobial activity of onion extracts against MTCC cultures revealed that all the three onion cultivars were highly active against *Staphylococcus aureus*, it showed more than 25 mm zone of inhibition. Among the three onion cultivars, O2 showed better antimicrobial activity against all the tested bacterial cultures.

Conclusion: This study showed that onion has good broad spectrum bioactivity against microorganisms revealed that it contains phytochemicals which can be used as nutraceuticals.

Keywords: Phytochemicals, High-performance thin layer chromatography, Onion, Quercetin, Antimicrobial activity, Microbial type culture collection cultures.

INTRODUCTION

Allium cepa Linn. (onion) is a member of the Liliaceae family with over 250 genera and 3700 species. These plants are able to survive in harsh conditions such as winter, dryness, due to their bulbs [1]. *Allium* species have a variety of pharmacological effect including antioxidant, antimutagenic, antibacterial, antiviral, anti-inflammatory, antithrombotic, antifungal, chemopreventive activity, anti-tumor activity, etc., [2-6]. All these pharmacological effects are due to the presence of phytochemicals. The major phytochemical in *Allium* species are flavonoids. The flavonoids occur in free-state and as glycosides. Three acetate units and phenylpropane units formed the naturally occurring phenols, flavonoids. They are extensively occurred in nature but are commonly present in young tissues. Flavonoids are referred to natural bioactive compound due to its inherent ability to change the reaction taking place in the body due to allergies, bacteria, virus, and carcinogens [7]. They are used broadly as chemotaxonomic markers. The flavonol quercetin, a phytoalexin are one of the most powerful bioactive compounds known. Currently, immense consideration is being given to determine efficiency, superiority and values of the plant raw material, and herbal formulations due to its incalculable health benefits [8]. Assessment of phytochemical is one of the tools for the quality determination of phytochemical screening, chemoprofiling, and analysis of marker compounds using modern analytical techniques. Estimation of these compounds is vital for recent research and an array of techniques are mandatory for this [9]. High-performance thin layer chromatography (HPTLC) method has emerged as a significant contrivance for the analysis of herbal formulations and natural drugs in the last two decades. This technique can be used qualitatively as well as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product [10]. The major advantage of this technique is that a number of samples can be analyzed concurrently with a small quantity of mobile phase [11]. Hence, this study is aimed to analyze the four different solvent extracts of onion cultivars collected from three different cultivation sites using HPTLC and to determine the antimicrobial activity of those extracts against 10 microbial type culture collection (MTCC) bacterial cultures.

METHODS

Collection of plant materials

A. cepa from different cultivation site such as Surandai (O1), Alankulam (O2) and Vilathikuklam (O3) were collected and brought to the laboratory for further analysis.

Processing of plant materials

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

Preparation of extracts

The organic solvents such as petroleum ether, chloroform, methanol, and distilled water were used for the extracting the bioactive compounds from *A. cepa* bulb. 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 (6 µl) were loaded on to the HPTLC plate at 8 mm bands length in the 10×10 silica gel 60 F_{254} thin layer chromatography (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 100 µl 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 70 mm. Densitometric scanning was performed with a CAMAG TLC scanner 3 in reflectance absorbance mode at 254 nm, under the 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 8 mm diameter was punched into the MHA medium and filled with 10-50 µl (100-500 µg) of solvent extract. The plates were incubated at 37°C for 24 hrs. After the 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 fingerprinting profile

TLC was performed to separate the bioactive compounds and to identify one of the phytochemical flavonoids in the extract. The developing system consists of chloroform: Methanol (8:2 v/v) gave a sharp and well-defined band for quercetin, a flavonoid. This confirmed the presence of bioactive compound flavonoid in the extracts. The identity of quercetin was confirmed by comparing the bands of standard quercetin with that of extracts and by comparing retention factor (Rf) of reference with the standard.

The petroleum ether extract of all the three cultivars of onion showed a band coincides with standard quercetin and the Rf value was found as 0.68. O1 was found with 2 more bands with Rf values of 0.62 and 0.48 in which one band matched with O3 (Rf value 0.62). One more band was identified in O2 (Rf value 0.83) and 2 bands in O3. These results showed that 2 more compounds other than quercetin were isolated in O1 and O3 onion cultivars (Fig. 1a).

The chloroform extract of onion showed a band matched with the standard quercetin (Rf - 0.68). All the three cultivars revealed one more band with approximately, similar Rf value (0.77) apart from quercetin. Additional band was observed in O1 with Rf value 0.89 (Fig. 1b). Quercetin was identified in methanol extract of all the three onion cultivars with Rf value of 0.66 which was confirmed by comparing the band pattern with that of standard quercetin. 5 more compounds were separated in O1 and O3 and 3 compounds in O2 in which one compound was found common in all the three onion cultivars (Fig. 1c). The Rf value of quercetin in water extracts of onion was 0.68, which was confirmed with standard quercetin. 6, 5, and 2 additional compounds were separated in O3, O2, and O1 in which one compound was found common in O2 and O3 (Fig. 1d). In HPTLC analysis of onion, more compounds were isolated in petroleum ether, methanol, and water extract of O3.

Antimicrobial activity of onion against MTCC cultures

The solvent extracts of onion; O1 exhibited good antibacterial activity against all the 10 MTCC cultures tested. Petroleum ether, methanol, and water extract showed maximum activity against *Staphylococcus aureus* (20.5±0.5 mm) and 25.33±0.29 mm respectively). Chloroform extract showed maximum activity against *Salmonella typhi* (18.5±0.5 mm). *Klebsiella pneumoniae, S. typhi, Aeromonas hydrophila* showed resistant to petroleum ether extract. *K. pneumonia, Pseudomonas aeruginosa* were resistant to chloroform extract. *S. typhi* was resistant to water extract of O1 (Table 1 and Fig. 2).

Water, methanol, and petroleum ether extract of O2 showed maximum activity against *S. aureus* (27±0.5 mm, 24.0.5±0.5 mm and 19.83±0.29 mm, respectively). Chloroform extract of O2 showed wide spectrum activity against *Bacillus subtilis* (19.83±0.29 mm). *K. pneumonia* showed less activity to petroleum ether. *Proteus vulgaris* was resistant to petroleum ether extract of O2. *S. typhi, Enterobacter aerogenes* and Streptococci showed a maximum sensitivity to methanol extract (20.33±0.58 mm, 18±0.5 mm, and 18.33±0.29 mm). *P. aeruginosa* and *A. hydrophila* exhibited good sensitivity to methanol and water extract (21.83±0.76 mm and 22.33±0.29 mm; 17.83±0.29 mm and 19.33±0.58 mm). *Escherichia coli* exhibited maximum sensitivity to chloroform, methanol, and water extract (Table 2 and Fig. 3).

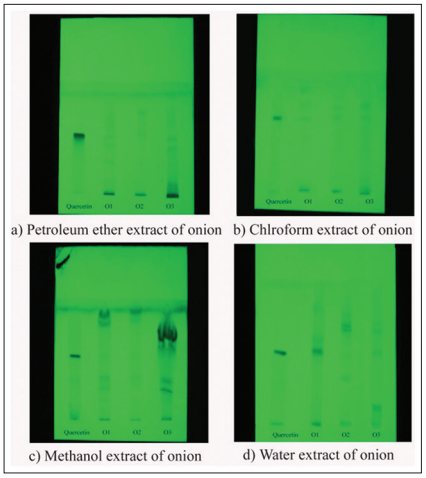

Fig. 1: High-performance thin layer chromatography fingerprinting of three onion cultivars, (a) Petroleum ether extract of onion, (b) chloroform extract of onion, (c) methanol extract of onion, (d) water extract of onion

Table 1: Antimicrobial activity of O1 (onion from Surandai) against MTCC cultures

MTCC cultures	Solvent extracts of O1 (500 µg)			
	Petroleum ether	Chloroform	Methanol	Water
K. pneumoniae	9.17±0.29	8.67±0.29	13.33±0.58	11.17±0.29
E. coli	15.5±0.5	16.5±0.5	12.5±0.5	14.5±0.5
S. pyogenes	11.17±0.29	16.5±0.5	17.5±0.5	16.5±0.5
P. aeruginosa	10.33±0.29	8.83±0.29	25.33±0.58	20.67±0.58
E. aerogenes	12.33±0.29	13.67±0.58	12.83±0.29	14.33±0.58
S. aureus	20.5±0.5	13.33±0.58	25.5±0.5	25.33±0.29
P. vulgaris	10.17±0.29	15.67±0.58	18.5±0.5	18.5±0.5
S. typhi	9.83±0.29	18.5±0.5	13.67±0.29	9.17±0.29
B. subtilis	13.83±0.29	16.83±0.29	11.17±0.29	12.5±0.5
A. hydrophila	9.17±0.29	17.17±0.29	14.83±0.76	17.5±0.87

*All values are expressed as mean±SEM for three determinations, MTCC: Microbial type culture collection, SEM: Standard error of mean, *K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, S. pyogenes: Streptococcus pyogenes, P. aeruginosa: Pseudomonas aeruginosa, E. aerogenes: Enterobacter aerogenes, S. aureus: Staphylococcus aureus, P. vulgaris: Proteus vulgaris, S. typhi: Salmonella typhi, B. subtilis: Bacillus subtilis, A. hydrophila: Aeromonas hydrophila*

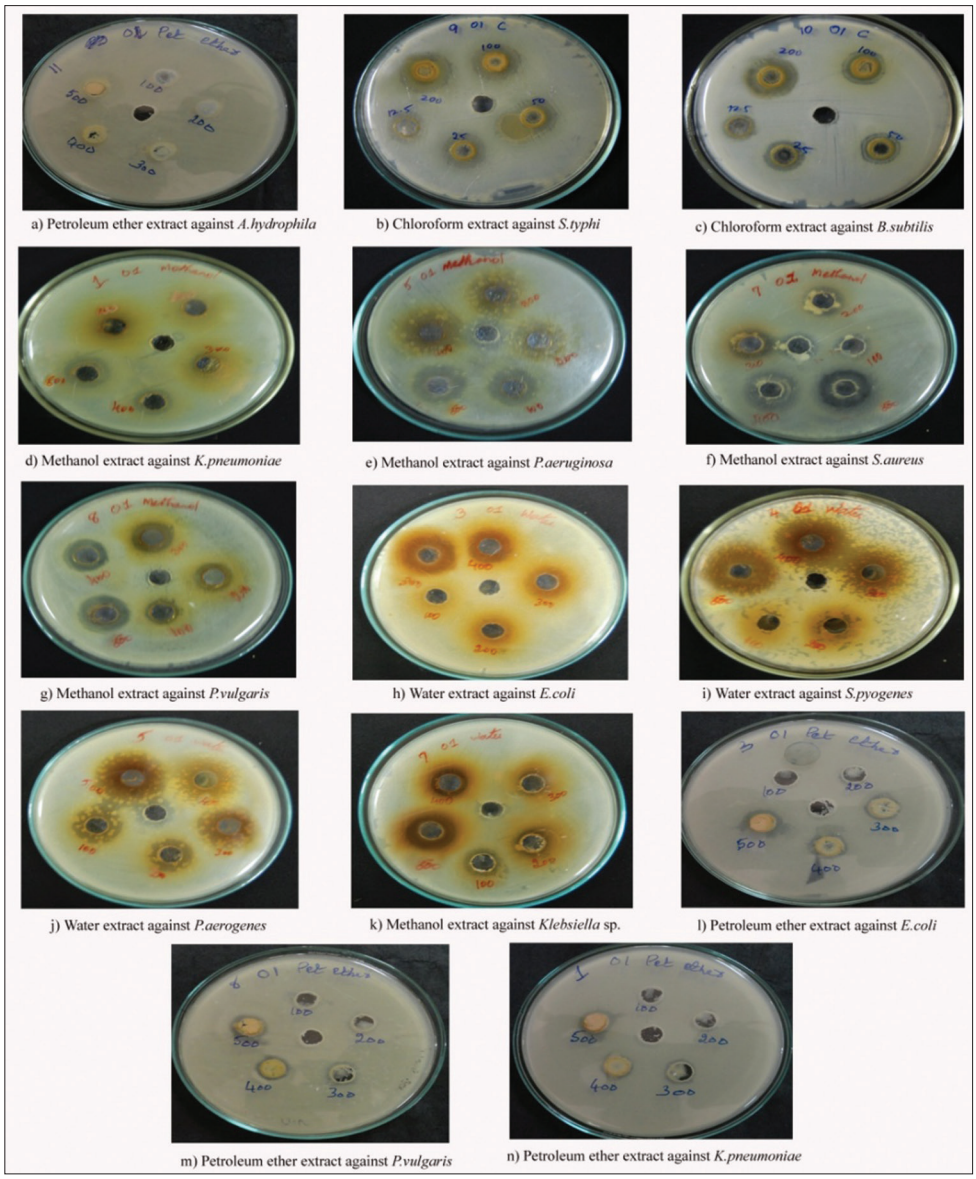

Fig. 2: Antimicrobial activity of O1 against microbial type culture collection cultures, (a) petroleum ether extract against *Aeromonas hydrophila*, (b) chloroform extract against *Salmonella typhi,* (c) chloroform extract against *Bacillus subtilis,* (d) methanol extract against *Klebsiella pneumoniae,* (e) methonal extract against *Pseudomonas aeruginosa,* (f) methonal extract against *Staphylococcus aureus,* (g) methanol extract against *Proteus vulgaris,* (h) water against *Escherichia coli,* (i) water extract against *Streptococcus pyogenes*, (j) water extract against *Pasteurella aerogenes,* (k) methanol extract *Klebsiella* sp., (l) petroleum ether extract against *Escherichia coli,* (m) petroleum ether against *P. vulgaris,* (n) petroleum ether extract against *K. pneumoniae*

All the four extracts of O3 showed wide spectrum activity against *S. aureus* (more than 18 mm). Petroleum ether extract of O3 showed less activity against *P. vulgaris* and *B. subtilis* (9.83±0.29 mm). Chloroform extract showed less activity against *K. pneumonia* and *P. vulgaris.* Methanol extract of O3 exhibited good antibacterial activity against all the 10 MTCC cultures tested (Table 3 and Fig. 4). In this study, methanol extract was found to be effective in inhibiting the bacterial organisms.

DISCUSSION

The peak area of quercetin concentration was recorded. 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*. [12]. 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*. [13]. 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*. [14] separated one specific sapogenin in garlic, β-chlorogenin, by TLC. In Parmar *et al*. [15] 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 results by Ye *et al*., [16] showed that the essential oil of onion exhibited a potent inhibitory effect against all bacteria (*E. coli,*

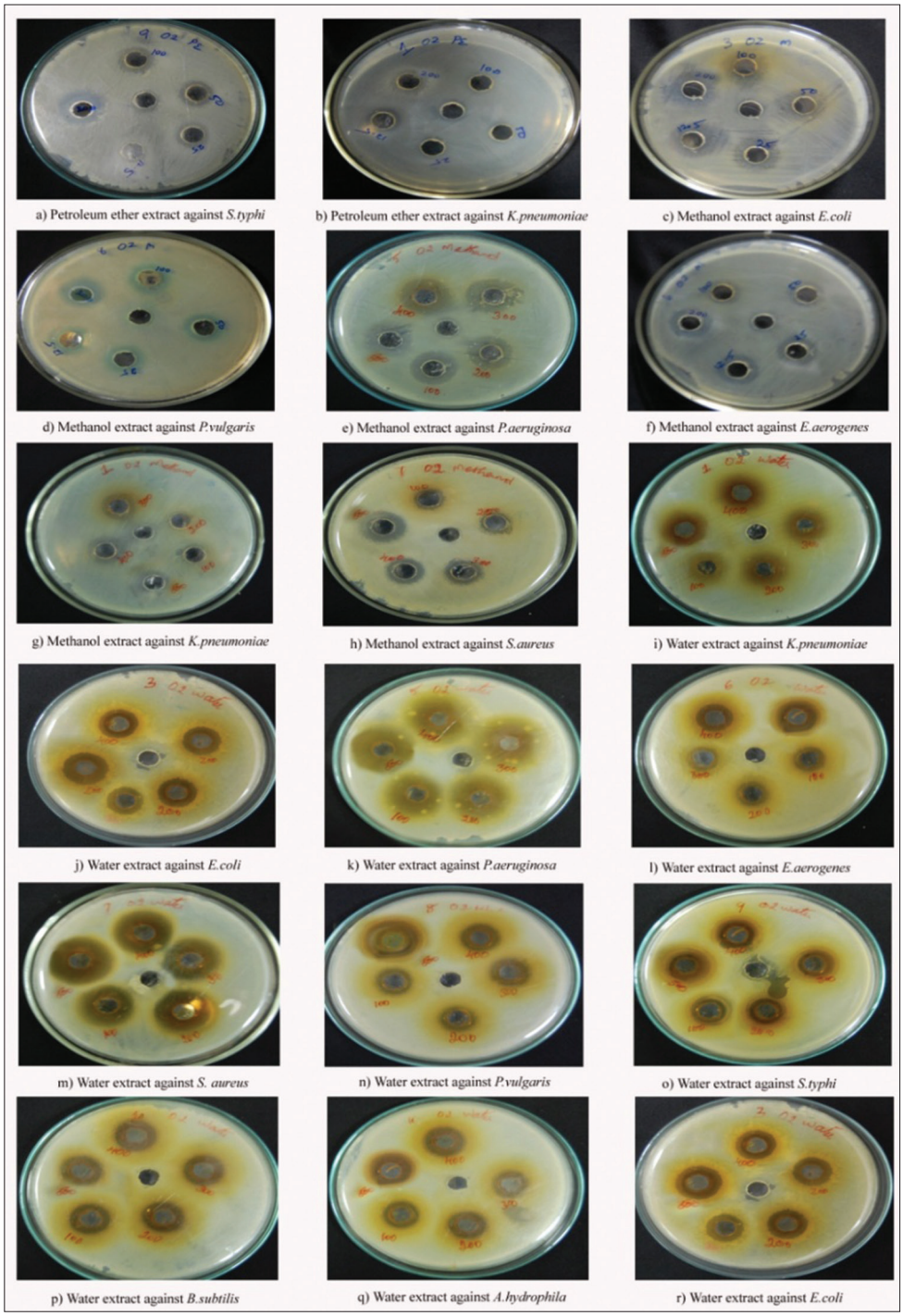

Fig.3: Microbial type culture collection, (a) petroleum ether extract against *Salmonella typhi*, (b) petroleum ether extract against *Klebsiella pneumoniae*, (c) methanol extract against *Escherichia coli*, (d) methanol extract against *Proteus vulgaris*, (e) methanol extract against *Pseudomonas aeruginosa*, (f) methanol extract against *Enterobacter aerogenes*, (g) methanol extract against *K. pneumoniae*, (h) methanol extract against *Staphylococcus aureus*, (i) water extract against *K. pneumoniae*, (j) water extract against *E. coli*, (k) water against *P. aeruginosa*, (l) water extract of *E. aerogenes*, (m) water extract against *S. aureus*, (n) water extract against *P. vulgaris*, (o) water extract against *S. typhi*, (p) water extract against *Bacillus subtilis*, (q) water extract against *Aeromonas hydrophila*, (r) water extract against *E. coli*

B. subtilis, and *S. aureus*) with diameter of inhibition zones ranging from 4.1 to 19.3 mm. The essential oil exerted a broad antimicrobial spectrum and showed a high antimicrobial effect on *B. subtilis*. The data obtained by Zohri *et al*., [17] indicated that Gram-positive bacteria were more sensitive to onion oil than Gram-negative bacteria. Onion oil was highly active against the four Gram-positive bacteria tested and only one isolate of Gram-negative bacteria (*Klebsiella pneumoniae*, 12 mm).

Adeshina *et al*. [18] reported 35±0.1 mm and 30±0.2 mm zone of inhibition against *P. aeruginosa* by white and red onion respectively. Furthermore, they reported 19±0.5 mm and 15±0.2 mm zone against *E. coli*, 35±0.2 mm,

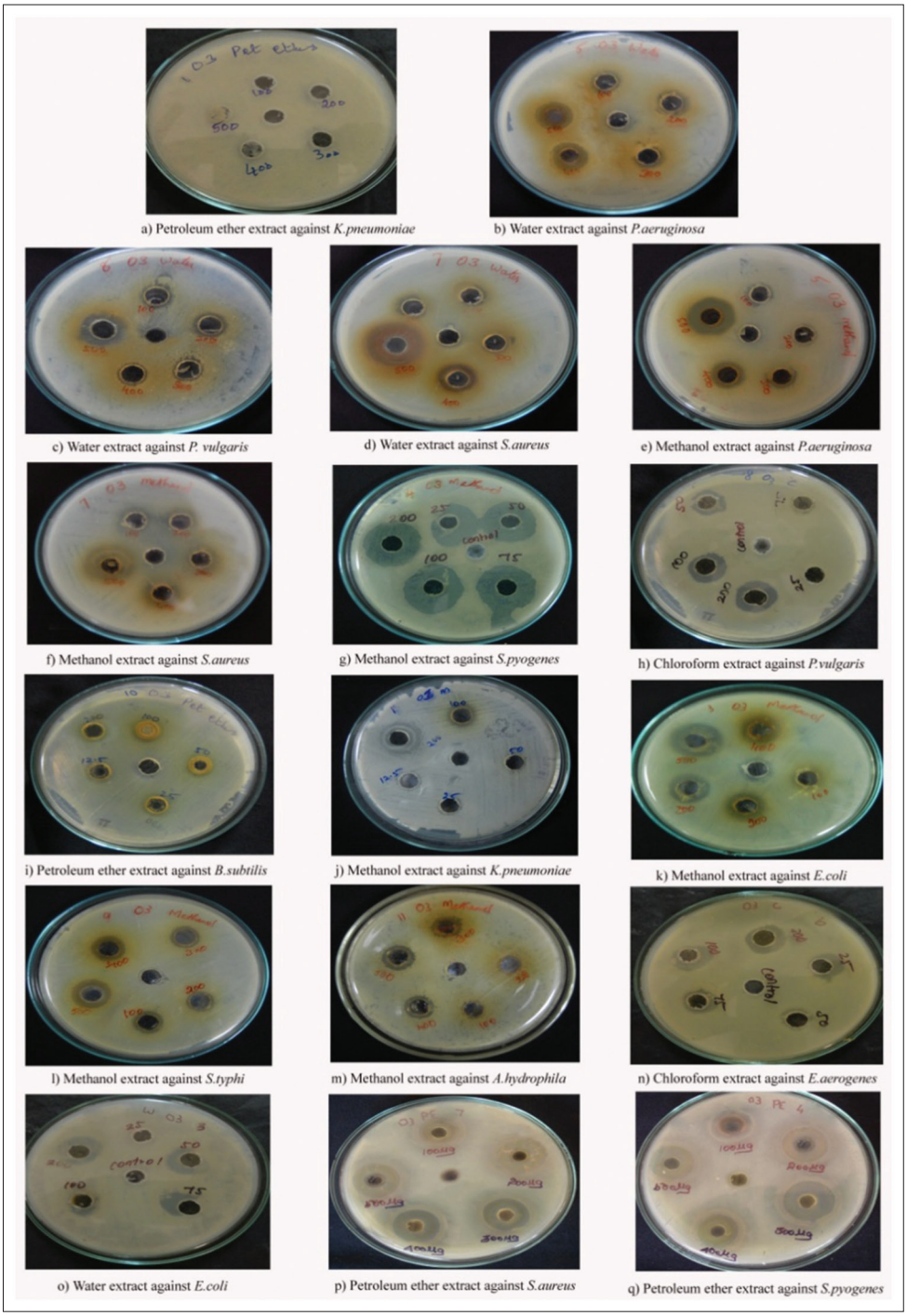

Fig. 4: Antimicrobial activity of O3 against microbial type culture collection cultures, (a) petroleum ether extract against *Klebsiella pneumoniae*, (b) water extract against *Pseudomonas aeruginosa*, (c) water extract against *Proteus vulgaris*, (d) water extract against *Staphylococcus aureus*, (e) methanol extract against *P. aeruginosa*, (f) methanol extract against *S. aureus*, (g) methanol extract against *Streptococcus pyogenes*, (h) chloroform extract against *P. vulgaris*, (i) petroleum ether extract against *Bacillus subtilis*, (j) methanol extract against *Klebsiella pneumoniae*, (k) methanol extract against *Escherichia coli*, (l) methanol extract against *Salmonella typhi*, (m) methanol extract against *Aeromonas hydrophila*, (n) chloroform extract against *Enterobacter aerogenes*, (o), water extract against *E. coli*, (p) water extract against *S. aureus*, (q) petroleum ether extract against *S. pyogenes*

and 28±0.1 mm zone against *S. typhi* by white and red onion, respectively. Among the non-polar and polar subfractions of methanolic extracts of three Spanish onion varieties assayed by Santas *et al.* [19], only non-polar subfractions showed good antimicrobial inhibition.

Shenoy *et al*., [1] reported that all these four solvent extracts showed good antimicrobial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *E. coli*. *A. cepa* extract was found ineffective against tested pathogens in Rekha and Shruti's [20] report.

CONCLUSION

In the present study, a valuable and easiest technique for the scrutiny of flavonoids in onion using HPTLC was effectively performed. This

Table 2: Antimicrobial activity of O2 (onion from Alankulam) against MTCC cultures

MTCC cultures	Solvent extracts of O2 (500 µg)			
	Petroleum ether	Chloroform	Methanol	Water
K. pneumoniae	9.83±0.29	10.83±0.76	16.17±0.29	12.67±0.58
E. coli	10.17±0.29	17.5±0.5	17.83±0.29	17.33±0.58
S. pyogenes	10.83±0.29	15.33±0.58	18.83±0.29	16±0.5
P. aeruginosa	10.17±0.29	13±0.5	21.83±0.76	22.33±0.29
E. aerogenes	15.17±0.29	14.83±0.76	18±0.5	15.33±0.58
S. aureus	19.83±0.29	15.33±0.58	24.5±0.5	27±0.5
P. vulgaris	8.83±0.29	14.5±0.5	13.5±0.5	18.5±0.5
S. typhi	15.17±0.29	18.5±0.5	20.33±0.58	11.67±0.58
B. subtilis	10.67±0.58	19.83±0.29	17.5±0.5	12.17±0.29
A. hydrophila	10.83±0.29	12.5±0.5	17.83±0.29	19.333±0.58

*All values are expressed as mean±SEM for three determinations, MTCC: Microbial type culture collection, SEM: Standard error of mean, *K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, S. pyogenes: Streptococcus pyogenes, P. aeruginosa: Pseudomonas aeruginosa, E. aerogenes: Enterobacter aerogenes, S. aureus: Staphylococcus aureus, P. vulgaris: Proteus vulgaris, S. typhi: Salmonella typhi, B. subtilis: Bacillus subtilis, A. hydrophila: Aeromonas hydrophila*

Table 3: Antimicrobial activity of O3 (onion from Vilathikulam) against MTCC cultures

MTCC cultures	Solvent extracts of O3 (500 µg)			
	Petroleum ether	Chloroform	Methanol	Water
K. pneumoniae	10.83±0.29	8.83±0.29	12.17±0.29	11.33±0.29
E. coli	11.5±0.5	14.33±0.29	16.16±0.29	14.17±0.29
S. pyogenes	16.33±0.29	15.33±0.29	19.5±0.5	17.17±0.29
P. aeruginosa	10.17±0.28	12.83±0.29	20.67±0.58	18.67±0.58
E. aerogenes	14.83±0.29	13.33±0.58	18.83±0.29	15.83±0.29
S. aureus	21.67±0.58	18.33±0.58	20.67±0.58	20.5±0.5
P. vulgaris	9.83±0.29	10.33±0.58	14.33±0.58	14.83±0.29
S. typhi	14.17±0.29	12.67±0.58	15.33±0.58	12.5±0.5
B. subtilis	9.83±0.29	15.67±0.29	14.17±0.29	10.83±0.29
A. hydrophila	10.83±0.29	13.5±0.5	15.17±0.29	12.5±0.87

*All values are expressed as mean±SEM for three determinations. MTCC: Microbial type culture collection, SEM: Standard error of mean, *K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli, S. pyogenes: Streptococcus pyogenes, P. aeruginosa: Pseudomonas aeruginosa, E. aerogenes: Enterobacter aerogenes, S. aureus: Staphylococcus aureus, P. vulgaris: Proteus vulgaris, S. typhi: Salmonella typhi, B. subtilis: Bacillus subtilis, A. hydrophila: Aeromonas hydrophila*

method is extremely responsive and reproducible technique to spot the phytochemicals with accuracy. Based on the antimicrobial activity of onion, onion from Vilathikulam was determined as the best cultivar since it showed finest result against the bacterial organisms. The make use of *Allium* sp. will diminish the side effects and cost coupled with the applications of synthetic antibiotics and will also be an eco-friendly measure. Since this medicinal plant, onion possess a lot of bioactive compounds, it can be a better and easily available material for nutraceuticals.

ACKNOWLEDGMENTS

We would like to thank Mr. R Anand for his help in collecting *Allium* species used in this study. We also express our gratitude to the principal and management of Udaya College of Arts and Science, Vellamodi (Tamil Nadu, India) for their moral support to carry out this research.

REFERENCES

1. Shenoy C, Patil MB, Kumar R, Patil S. Preliminary phytochemical investigation and wound healing activity of *Allium cepa Linn.* (*Liliaceae*). Int J Pharm Pharm Sci 2009;2(2):167-75.
2. Pratt DE. Natural antioxidants from plant material. In: Ho CT, Lee CY, Huang M, editors. Phenolic Compounds in Food and their Effects on Health II; ACS Symposium Series 507. Washington, D.C: American Chemical Society; 1992. p. 54-71.
3. Duthie GG, Duthie SJ, Kyle JA. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr Res Rev 2000;13(1):79-106.
4. Stajner D, Milic N, Canadanovic-Brunet J, Kapor A, Stajner M, Popovic BM. Exploring *Allium* species as a source of potential medicinal agents. Phytother Res 2006;20(7):581-4.
5. Nencini C, Cavallo F, Capasso A, Franchi GG, Giorgio G, Micheli L. Evaluation of antioxidative properties of *Allium* species growing wild in Italy. Phytother Res 2007;21(9):874-8.
6. Sofija D, Vanja T, Silvana P, Jelena KM, Silva D, Marina M, *et al*. Bioactivity assays on *Carlina acaulis* and *C. acanthifolia* root and herb extracts. Dig J Nanomater Biostruct 2012;7(3):1213-22.
7. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica* leaves from Ibadan, Oyo state. Plant Sci Res 2009;2(1):11-3.
8. Nagore DH, Patail PS, Kuber VB. Comparison between high performance liquid chromatography and high performance thin layer chromatography determination of Diosgenin from *Fenugreek* seeds. Int J Green Pharm 2012;6(4):315-20.
9. Jain A, Lodhi S, Singhai AK. Simultaneous estimation of quercetin and rutin in *Tephrosia purpurea* Pers by high performance thin-layer chromatography. Asian J Tradit Med 2009;4(3):104-9.
10. Chakraborthy GS, Ghorpade PM. Determination of quercetin by HPTLC in *Calendula officinalis* extract. Int J Pharma Bio Sci 2010;1(1):1-4.
11. Sethi PD. HPTLC. 1st ed. New Delhi: CBS Publishers and Distributors; 1996. p. 39.
12. Mishra J, Singh RD, Jadon VS, Gusain MP, Aradhana P. HPTLC profile of quercetin content of common bean (Uttarakhand) landraces growing in Uttarakand. Am J Food Tech 2012;7(2):96-100.
13. Bandyopadhyay C, Srirangarajan AN, Sreenivasan A. Studies of flavour components of onion (*Allium cepa*). I. Thin-layer chromatographic investigation of onion. J Chromatogr 1970;47(3):400-7.
14. Itakura Y, Ichikawa M, Mori Y, Okino R, Udayama M, Morita T. How to distinguish garlic from the other *Allium* vegetables. Recent advances on the nutritional effects associated with the use of Garlic as a supplement. Am Soc Nutri Sci 2001;963s-7.
15. Parmar S, Shah N, Kasarwala M, Virpura M, Shah K, Patel P. Determination of quercetin by HPTLC method present in zymodyne syrup: A poly herbal formulation. Int J Pharm Sci Res 2011;2(10):2724-8.
16. Ye CL, Dai DH, Hu WL. Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa L*). Food Control 2013;30(1):48-53.
17. Zohri AN, Abdel-Gawad K, Saber S. Antibacterial, anti dermatophytic and antitoxigenic activities of onion (*Allium cepa L*) oil. Microbiol Res 1995;150(2):167-72.
18. Adeshina GO, Jibo S, Agu VE, Ethinmidu JO. Antibacterial activity of fresh juices of *Allium cepa* and *Zingiber officinale* against multidrug resistant bacteria. Int J Pharm Biosci 2011;2(2):289-95.
19. Santas J, Pilar MA, Carbo R. Antimicrobial and antioxidant activity of crude onion (*Allium cepa L.*) extracts. Int J Food Sci Technol 2010;45(2):403-9.
20. Rekha J, Shruti D. Phytochemical extraction and antimicrobial activity of some medicinal plants on different microbial strains. J Med Plants Stud 2014;2(3):58-63.