
High performance thin layer chromatography analysis of bioactive components from *Curcuma longa*; an anti-microbially effective medicinal spice

Kurhekar Jaya Vikas

Department of Microbiology, Bharati Vidyapeeth's, Dr. Patangrao Kadam Mahavidyalaya, Sangli, Maharashtra, India

Email address:

jaya_kurhekar@rediffmail.com

To cite this article:

Kurhekar Jaya Vikas. High Performance Thin Layer Chromatography Analysis of Bioactive Components from *Curcuma longa*; an Anti-Microbially Effective Medicinal Spice. *American Journal of BioScience*. Special Issue: Pharmacognosy with reference to evaluation of medicinal plants as an alternative therapy. Vol. 3, No. 1-1, 2015, pp. 1-6. doi: 10.11648/j.ajbio.s.2015030101.11

Abstract: *Curcuma longa* - haladi, a plant grown as a spice is an entity known to have significant antimicrobial potency. This study was carried out to analyze its bio-active components, with the help of High Performance Thin Layer Chromatography (HPTLC). Anti-microbial effects of *C. longa* were checked using aqueous and acetone extracts, against a few bacterial pathogens causing common infections. The aqueous extract was found effective in inhibiting the growth of eight pathogens (64% efficiency) while the acetone extract in inhibiting the growth of three pathogens (21% efficiency). Flavonoid analysis of aqueous extract by HPTLC showed peaks probably corresponding to Coumarins, Triterpenoids, Echinacosides, Eriodictyl, Cichoric acid and Caffeic acid derivatives, responsible for its anti-microbial activities. This natural bio resource may prove to be of significance in the armamentarium of antimicrobial agents.

Keywords: *Curcuma longa*, Antimicrobial Activity, Bio-Active Components, MIC, HPTLC

1. Introduction

Indian medicinal plants, constituting a major section of plants in the whole world, are of great importance in the modern scenario, India being one of the richest plant based ethno-medicinal traditions in the world (Baskar and Chezhiyan, 2002). Plants are a source of medicinal agents and raw materials for manufacture of drugs, 1/3rd of the pharmaceuticals being of plant origin. WHO currently encourages, recommends and promotes the inclusion of herbal drugs in national health care programs. (Khan and Khanum, 2002). Maharashtra, because of its geographical location and suitable climate, is fit for cultivation of majority of commercially important medicinal crops. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections. Alkaloids and flavonoids present in many fruits, vegetables have a major role in prevention of several forms of cancers and cardiovascular diseases. Medicinal and Aromatic plants as natural resources are being used up at a high rate and thus have to be maintained and preserved.

Rhizome of *Curcuma longa* is externally used as a powder, paste, lotion or oil, ointment, decoction. It is known to relieve the symptoms of measles, chicken-pox, URTI and cold (Sathe, 1998)

It is known to be a blood purifier and consumed along with neem fruits, in case of scabies and itching. Worms are traditionally treated with haladi and jaggery. It is used in cases of excessive incontinence, jaundice, cold and cough, poisoning, conjunctivitis, wounds, piles, peri and post-delivery as an anti-septic (Sawant, 1974). Fresh haladi contains curcumin, alkalies, essential (anti-septic) oil, ketone, alcohol, zingiberine and carmin (antacid) (Sathe, 1998) extracts (Sastri, 1952) have been reported as antioxidants of non-enzymatic category, known to exhibit significant anti-inflammatory and potential antioxidant activity (Salimath et al, 1986; Chawla et al, 1987). Curcuminoids, a group of phenolic compounds isolated from the roots of *C. longa*, exhibit a variety of beneficial effects on health and prevention of diseases (Joe et al, 2004). A study using RT-PCR showed that all three curcuminoids isolated from *C. longa* inhibited multidrug resistance - 1 (MDR-1) gene expression (Limtrakul et al, 2004); Wound healing and detoxifying properties of curcumin have received

considerable attention (Bone, 1991; Kaushik and Dhiman, 2000; Joe et al, 2004). Its anti-oxidant activity has been proved by Lipid Peroxidation assay and shown to inhibit lipid peroxidation. Scartezzini and Speroni, 2000 ; Ciddi and Kaleab, 2005). It is being used as one of the ingredients of a herbal facial scrub and found comparable with popularly used marketed scrub, showing excellent effects on acne, healing and prevention of pimples, giving fairness, smoother complexion, helping exfoliation (Rawat et al, 2005). Thus, rhizome of *C. longa* has been used as a medicine, spice and coloring agent for thousands of years.

With this perspective, present study was carried out to analyze the bio-active components, in the rhizome of *C. longa*, with the help of High Performance Thin Layer Chromatography.

2. Materials and Methods

Preparation of plant extracts:

For preparation of extracts, fresh rhizomes of *C. longa* have been used.

a) **Aqueous extract:** Known weight of rhizomes were washed with a known volume of sterile distilled water and crushed in a grinder, till a very fine paste. The material was then filtered through a muslin cloth and Whatman no.40 filter paper. The fine powder obtained on shade drying was used as the sample for investigation (Bambode and Shukla, 1973).

b) **Acetone extract:** Known weight of rhizomes was washed with a known volume of sterile distilled water and crushed in a grinder using a known amount of acetone. The mixture was refluxed at 60 °C for one hour. The extract was filtered through muslin cloth and Whatman no. 40 filter paper. Acetone was allowed to evaporate and the powder thus obtained was used as a sample (Shekhawat and Prasada, 1971).

2.1. Microorganisms used

Twelve clinical isolates, four Gram positive and eight Gram negative, were used for this study.

Gram positive: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus species*, *Micrococcus luteus*.

Gram negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Salmonella paratyphi B*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Serratia marsescens*.

Detection of anti-microbial activity of the extracts: was carried out by agar-cup diffusion assay method (Finegold and Baron, 1986).

Detection of sensitivity of the isolates to antibiotics: was carried out by disc diffusion assay method (Bauer et al, 1966).

2.2. Antibiotics Used

For sensitivity testing, antibiotic octadises number OD 042 from Himedia have been used to check the response of the isolates and standard cultures. Most commonly prescribed antibiotics were used; Amikacin, Augmentin, Cephotaxime,

Ciprofloxacin, Cefuroxime, Cefoperazone, Ceftazidime, Gentamicin, Lomefloxacin, Netilmicin, Ofloxacin, Pefloxacin, Nalidixic acid, Nitrofurantoin, Norfloxacin, Doxycycline and Chloramphenicol

2.3. Qualitative Analysis of the Effective Extracts by HPTLC

High Performance thin Layer Chromatography (HPTLC) was carried out to verify the presence of constituents in the herbal extract under investigation. Analysis of components was done on the basis of R_f ie Retention factor values (Gangwar and Kumar, 2006). (Graphs and Tables). Flavonoid analysis was done using;

Instrument: CAMAG TLC Scanner 3 “Scanner3_070408” S/N 070408 (1.14.21),

Stationary phase: TLC Al sheet Silica gel 60 F254 precoated MERCK Cat. No. 1.05554 of size 10 x 10 cm., Mobile phase: Chloroform : Acetone : Formic acid :: 7.5 : 1.7 : 0.4.

Sample application volume: 5 µl. of each sample was applied on precoated layer 10 mm from the bottom edge, band length 8 mm, distance between the tracks 17.5 mm, distance from the sides 15 mm, Chamber saturation: 0 min, Developing distance: 80 mm, Measurement mode: UV Absorbance / Reflectance, Development chamber: Camag Twin Trough Chamber 10 X 10 cm with S. S. lid, Developing reagent / Derivatizing agent: Natural products / Polyethylene Glycol No. 28.

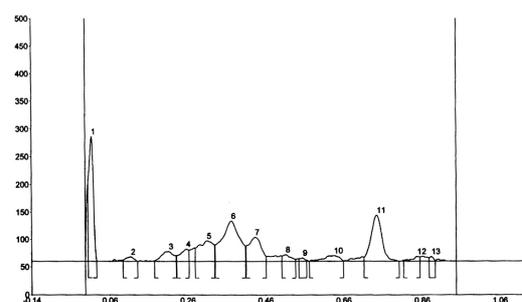
Analysis of components was done on the basis of R_f ie Retention factor values (Gangwar and Kumar, 2006). All the peaks were identified by referring to “Plant drug analysis, A thin layer chromatography, Atlas” by Wagner and Bladt (1996).

2.4. Statistical Analysis

The data was analyzed by simple arithmetic means of the diameters of zones of inhibition obtained after repeating the antimicrobial assay three times. The standard error was compared to the control. Statistical analysis of the data was done by applying statistical unpaired t-test. (Mahajan B. K, 1989)

3. Observations and Results

Track 3, ID: CL : 30 µl 200 nm.



Graph 1. (HPTLC chromatogram of *Curcuma longa* - 200 nm)

Table I. Effect of extracts of *Curcuma longa* on common isolates

Name of the Culture	Aqueous extract	Acetone extract
	Mean ± S.D.	Mean ± S.D.
<i>B.subtilis</i>	19.33 ± 1.63	-
<i>S. aureus</i>	18.66 ± 1.36	-
<i>E. fecalis</i>	19.66 ± 1.21	15.66 ± 1.03*
<i>Micrococcus luteus</i>	-	19 ± 1.41
<i>E. coli</i>	16.5 ± 1.37	-
<i>K.pneumoniae</i>	-	-
<i>S. typhi</i>	17.83 ± 1.94	-
<i>S paratyphi B</i>	-	-
<i>S. flexneri</i>	16.5 ± 1.04	-
<i>P. aeruginosa</i>	15.33 ± 1.50	-
<i>P. vulgaris</i>	19.5 ± 1.04	-
<i>S. marsecens</i>	-	17.5 ± 1.04
Total no. of orgs. responding	8	3

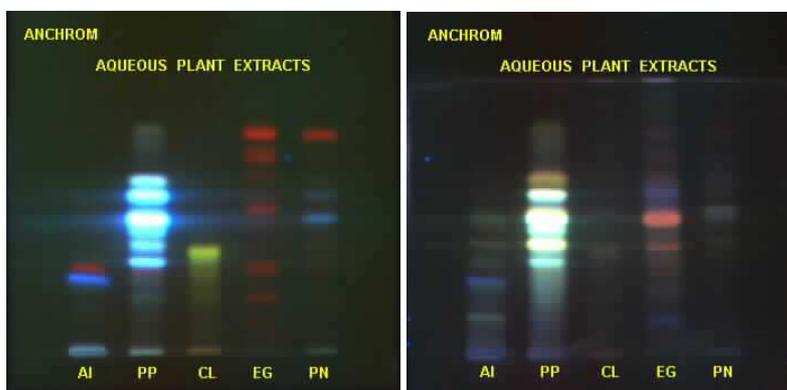
Key: Values are mean ± S.E.M. (n=3), significance Vs control; * P < 0.05, significant, ** P < 0.01 highly significant, *** P < 0.001, very highly significant, “-” refers to no antibacterial effect of medicinal plant to the mentioned bacterial strain at mentioned dose.

[Zone sizes compared with those of standard antibiotics as referred to in the Zone interpretive chart of standard antibiotics]

Table II. Analysis of *Curcuma longa* extract by HPTLC (200 nm)

Peak (200 nm)	Rf value	Area	Relative conc. %	Assigned component
2.	0.11	136.5	1.25	Coumarins
4.	0.25	391.7	3.60	Triterpenoids
9.	0.55	69.9	0.64	Triterpenoids

Mobile phase: Toluene : ethyl acetate : formic acid (100%) :: 7 : 3 : 0.2,



Before derivatisation

After derivatisation

Plate I. HPTLC Chromatograms of aqueous extracts (366 nm)

Key: CL = *Curcuma longa*

4. Discussion

C. longa is used everyday in Indian kitchens to impart color, flavor and a typical taste to vegetable preparations. It is known to be an antiseptic, anti-inflammatory agent and used in case of emergencies as a dressing. It is used as a gargle in case of throat infections.

In the present study, rhizomes of *C. longa* were extracted in distilled water (17 gm %) and acetone (44 gm %). 0.1 ml.

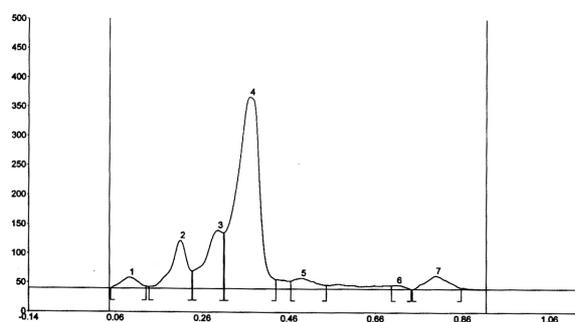
Derivatising agent : 5% Methanolic sulphuric acid.

Table III. Analysis of *Curcuma longa* extract by HPTLC (366nm)

Peak (366 nm)	Rf value	Area	Relative conc. %	Assigned component
1.	0.09	606.3	2.69	Echinacoside
2.	0.21	2450.3	10.86	Caffeic acid derivative
3.	0.29	3340.7	14.81	Eriodictyl
4.	0.37	14226.7	63.06	Caffeic acid derivative
5.	0.49	803.4	3.56	Caffeic acid derivative
6.	0.71	165.2	0.73	Caffeic acid derivative
7.	0.80	969.2	4.30	Cichoric acid

Mobile phase : Toluene : ethyl acetate : formic acid (100%) :: 7 : 3 : 0.2, Derivatising agent : 5% Methanolic sulphuric acid.

Track 3, ID: CL 366 nm.



Graph II. HPTLC chromatogram of *Curcuma longa* (366 nm)

of each extract was then tested against each isolate, for antimicrobial activity, using bioassay method.

Fourteen lab isolates were used to study the antimicrobial activity of crude aqueous and acetone rhizome extracts of *C. longa*. The aqueous extract was found effective in inhibiting the growth of eight organisms. Gram positive strains were *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus fecalis* while *Micrococcus luteus* was found to be resistant. Of the Gram negative isolates, *Escherichia coli*, *Salmonella*

typhi, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Proteus vulgaris* were found to be susceptible (Table I). The acetone extract was found effective in inhibiting the growth of three organisms. Gram positive strains were *Enterococcus faecalis* and *Micrococcus luteus*, Gram negative isolates *Serratia marsescens* (Table I).

Bacillus subtilis was found to be most sensitive to the aqueous extract while *Micrococcus luteus* to acetone extract. *Pseudomonas aeruginosa* was least sensitive to the aqueous extract and *Enterococcus faecalis* to acetone extract, as concluded from the zone sizes. Comparative efficiencies of aqueous and acetone extracts in inhibiting *Enterococcus faecalis* were statistically significant. The aqueous extract of *C. longa* showed 64% efficiency while acetone extract showed 21% efficiency. Aqueous extract showed more than 50% efficiency and acetone extract of *C. longa*, less than 50% efficiency. In general, aqueous extract is found better than acetone extract and can be used as a broad spectrum antibiotic to restrict the growth of common pathogens. Aqueous extract of *C. longa* inhibited eight of the fourteen isolates, exhibiting same potency as Gentamicin, Cephalexin, Netillin, Ofloxacin and Pefloxacin, in its antimicrobial action.

The aqueous extract of *C. longa* extract at 200 nm showed peaks probably corresponding to coumarins (1.25%) and triterpenoids (3.6%, 0.64%) and at 366 nm, the presence of echinacosides (2.69%), eriodictyl (14.81%), cichoric acid (4.3%) and caffeic acid derivatives (10.86%, 63.06%, 3.56%, 0.73%).

The clinical bacteriological study of *C. longa* was effective in case of conjunctivitis (Shrinivas, and Prabhakaran, 1987). The aqueous extract of *C. longa* is effective against pathogens isolated from wounds, *Actinomyces* species and two unidentified species. Rhizomes of *C. longa* are considered to have natural antibacterial, anti-inflammatory, antineoplastic and analgesic activities because they contain a number of monoterpenoids, sesquiterpenoids, and curcuminoids (Tang and Eisenbrand, 1992). The extracts of *C. longa*, at concentrations of 5-10 mg/ml, greatly reduced in vitro aflatoxin production by more than 90%, in case of *Aspergillus parasiticus* (Soni et al, 1992). The antibacterial activity of *C. longa* stem and root extract was reported and was effective against *S. aureus*, *E. coli* and a wide variety of pathogenic bacteria (Chandi et al, 1999). Antimicrobial properties of essential oils of *Curcuma longa* studied in vitro against *S. typhi*, *Kl. pneumoniae*, *E. coli*, *S. aureus* and *B. subtilis*, in different concentrations have showed antimicrobial activity against all but maximum activity against *E. coli* (Dubey et al, 2005). All these studies support the investigation carried out.

Banginwar and Tambekar (2003) reported that active principles like tannin, aliphatic ketones, beta-carotenoids, fatty acids, essential oils and phenolic compounds in the extract influence the anti-bacterial activity. Many medicinal plants owe their physiological activities, molecular interactions between alkaloid molecules and chemically defined components of the affected organisms, to their

content of alkaloids (Havsteen, 1983). Antioxidant principles from natural resources provide enormous scope in correcting the imbalance between pro-oxidant and antioxidant homeostasis responsible for diseases (Tiwari, 2001). Free radical scavenging activity has been ascribed to natural compounds, flavonoids and polyphenolics (Clark et al, 1985). Flavonoids exert multiple biological effects such as anti-inflammatory, anti-oxidant, anti-allergic, anti-viral and anti-malignancy chemotherapy (Havsteen, 1983). Flavonoids, phenolic compounds are present to a great extent in many fruits and vegetables and have a major role in prevention of several forms of cancer, cardiovascular diseases, cytotoxicity of LDL and are hydrogen donating free radicals with essentially the presence of free hydroxyl Groups and catechol moiety (Shetgiri and D'Mello, 2003). Tannins and alkaloids, plant secondary metabolites, are known to possess antimicrobial activity (Cowan, 1999). Flavonoids and alkaloids have been found to be responsible for antimicrobial activity. Quercetin, Rutin are flavonols (Jadhav and Kharya, 2005) showing very good free radical scavenging activity, flavon-3-ols like Catechin too reduce free radicals (Shetgiri and D'Mello, 2003). Components detected in *C. longa* by HPTLC have also been reported as very active molecules. Eriodictyol may be a good prototype for the development of more effective and/or more potent agents for the treatment of pain (Mateus et al, 2013). The coumarins are of great interest due to their pharmacological properties. In particular, their physiological, bacteriostatic and anti-tumor activity makes these compounds attractive and novel therapeutic agents (Jain and Joshi, 2012) Six new triterpenoids (1-6) were isolated from a *Penares* sp. sponge collected from Vietnamese waters. Triterpenoid 6 was cytotoxic against human leukemia HL-60 cells (IC₅₀ = 9.7 μM) (Kolesnikova et al, 2013). Echinacoside is one of the major active phenyl ethanoid glycosides in famous traditional Chinese medicine, with various bioactivities, such as antioxidation, neuroprotection, and hepatoprotection (Cunqin Jia et al, 2008). Chicoric acid properties have been reported to include anti-cancer, anti-obesity, antiviral, and anti-diabetic (King and Robinson, 1998; Pluymers et al., 2000; Charvat et al., 2006; Queffelec et al., 2008; Tusch et al., 2008; Tsai et al., 2012; Azay-Milhau et al., 2013; Xiao et al., 2013). Chicoric acid and its analogs have been claimed to possess anti-Human Immunodeficiency Virus (HIV) activity due to its involvement in HIV integrase inhibition, which could perhaps hinder HIV strain replication (King and Robinson, 1998; Charvat et al., 2006; Queffelec et al., 2008). Caffeic acid and some of its derivatives such as caffeic acid phenethyl ester (CAPE) and octyl caffeate are potent antioxidants which present important anti-inflammatory actions. Caffeic acid derivatives exert in vitro and in vivo anti-inflammatory actions (da Cunha FM et al, 2004). Looking at the efficacy of the *C. longa* extracts in inhibiting common pathogens and it being the store house of active components, known to have multiple health benefits, it can be concluded that *C. longa* may be included as an effective antimicrobial agent in the armamentarium of natural agents. If this natural medicinal

resource is managed efficiently with reference to its protection, conservation, cultivation and propagation, it will continue to prove beneficial to mankind.

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