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Evaluation of curcumin compound from methanolic extracts of *Curcuma longa* by high performance thin layer chromatography

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Abstract
Curcuma longa (turmeric) is a medicinal plant belonging to the family Zingiberaceae. It is widely used for the health care of humans, livestock and poultry. Turmeric is a potent antioxidant, anti-inflammatory, antimutagenic, antimicrobial, and anticancer agent. Curcumin is a polyphenol extracted from roots of turmeric. In the present study, fresh roots of *Curcuma longa* was collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu and evaluated for the identification and relative quantification of curcumin by using a simple, sensitive and accurate High Performance Thin Layer Chromatography method.

Keywords: Curcumin, turmeric, polyphenol, high performance thin layer chromatography

Introduction
Medicinal plants are widely used for curing various diseases in animal kingdom. Phytochemicals play a major role in plant defence mechanisms against predators and pathogens (Divisha *et al*., 2018) [6]. They are majorly classified into carotenoids and polyphenols which include phenolic acids, flavonoids, stilbenes and lignans (Molyneux *et al*., 2007) [8]. *Curcuma longa*, commonly known as Turmeric, a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberaceae, which is native to tropical South Asia. It is a medicinal plant that has a very long history of medicinal use, dating back nearly 4000 years. In Southeast Asia, turmeric is used not only as a principal spice but also as a component in religious ceremonies. Because of its brilliant yellow color, turmeric is also known as "Indian saffron". The rhizome, from which the turmeric is derived, is tuberous, with a rough and segmented skin. In Ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones, and relieving arthritis. Many South Asian countries use it as an antiseptic for cuts, burns, and bruises, and as an antibacterial agent (Benzie and Wachtel-Galor, 2011) [3].
Curcumin (diferuloylmethane), a water-insoluble polyphenol extracted from the herb *Curcuma longa L.* has been widely used in traditional Indian and Chinese medicine as well as in the food industry. This polyphenol has been demonstrated for several types of biological and pharmacological activities, including anti-inflammatory, anti-oxidant and anti-cancer properties, etc. (Mishra *et al*., 2017) [7]. This compound is the most important active ingredient responsible for the biological activity of turmeric It is the natural yellow pigment present in the roots of turmeric (Badreldin *et al*., 2006) [2]. Chemical synthesis of curcumin analogues has resulted in compounds with stronger anti-oxidant and cancer chemoprotective activities (Youssef *et al*., 2004) [11]. Curcumin protects human keratinocytes from xanthine oxidase injury by its anti oxidant activity (Bonte *et al*., 1997) [4]. Several studies have estimated phenol contents in medicinal plants using Thin Layer Chromatography (Ranganathan and Punniamurthy, 2013) [10] and using High Performance Thin Layer Chromatography (Aftab *et al*., 2015) [1]. In the present study, a sensitive, simple and accurate HPTLC method was established to identify and evaluate curcumin from turmeric sample cultivated at herbal garden of Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu.

Materials and Methods

Plant Source

Fresh roots of *Curcuma longa* was collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu. The roots were cleaned, shade-dried and powdered for further analysis.

Preparation of methanolic extract

25g of the powdered sample was extracted with 125ml of methanol for 6 hours in a Soxhlet apparatus (Uma *et al*., 2009). The extracts were subjected to Rotary evaporator to allow the solvent to evaporate completely. Standard curcumin was purchased from Sigma Aldrich (USA). All the solvents used in the study were of HPLC grade.

Identification of curcumin from turmeric using High Performance Thin Layer Chromatography (HPTLC)

The chromatographic procedure was carried out with silica gel 60 F254 Thin Layer Chromatographic (TLC) plate (Merck). Seven different volumes-1,2,3,4,5, 6 and 7µL of standard curcumin solution (1 mg/mL) and duplicates of 2, 4, 6 and 8 µL of turmeric samples (50 mg/ml) were applied to TLC plates by using CAMAG Linomat 5 sample applicator. Type of the application was banding and applied on Y axis of the plate with 8.0mm distance and the length was also maintained at 8.00 mm distance. The plate was then developed at room temperature in a CAMAG twin-trough vertical development chamber (20x10) already saturated for 20 min with the mobile phase of toluene: acetic acid (8:2 v/v). The solvent front position was maintained at 70 mm. After the development process, the plate was subjected to CAMAG visualizer2 for screening the developed plate using tungsten light source at a wavelength of 540 nm, maintaining a scanning speed of 20 mm/s and slit dimension of 6.0 mm x 0.45 mm. The scanning procedure was carried out using vision CATS software (version 2.5.18262.1). The detection of curcumin present in turmeric samples was observed on the HPTLC plates in the form of bands that held similar Retardation factor (Rf) values as that of curcumin standard. The Retardation factor (Rf) which indicates the ratio of the distance travelled by the compound to the distance travelled by the solvent in a given time was measured for both standard and sample spots.

Relative quantification of Curcumin in turmeric samples

The area calibration was measured for substance curcumin @ 540nm using CAMAG scanner (S/N: 250410). Four reference (Standard) samples and five turmeric samples were subjected for creating standard curve for the evaluation of curcumin from turmeric samples.

Results and Discussion

The table 1 showed the Rf values analysed by High Performance Thin Layer Chromatography (HPTLC) for curcumin standard and turmeric samples indicating the presence of curcumin in the turmeric samples. Fig.1 and fig.2 showed development of TLC plate with remission of 540 nm and identification of curcumin in turmeric sample with Rf value, respectively. Several research works utilized HPTLC for identification and evaluation of medicinal phytochemistry in view of its sensitivity and accuracy. Mishra *et al*. (2017) [7] also identified curcumin in plasma samples by using HPTLC method. Corina *et al* (2019) [5] analysed iridoids from *Lamium album* using HPTLC methods. Principal component was analysed for root extracts of *Withania coagulans* by using this chromatogram (Preethi *et al*., 2014) [9]. Studies so far conducted with CAMAG HPTLC used win CATS software whereas the present study was conduted with the sophisticated recent software known as vision CATS software. Moreover, no reports are available with regard to evaluation of curcumin from turmeric cultivated at Thanjavur district of Tamil Nadu.

Table 1: HPTLC –Curcumin profile of methanolic extract of turmeric samples (curcumin-1mg/ml; turmeric samples-50mg/ml)

Track	Rf	Height	Area	Assigned substance
Curcumin standard	0.397	0.1700	0.0040	Curcumin
Curcumin standard	0.384	0.1665	0.0038	Curcumin
Curcumin standard	0.384	0.1994	0.0055	Curcumin
Curcumin standard	0.385	0.2188	0.0063	Curcumin
Curcumin standard	0.384	0.2336	0.0065	Curcumin
Curcumin standard	0.381	0.2408	0.0071	Curcumin
Curcumin standard	0.384	0.2051	0.0063	Curcumin
Turmeric sample	0.387	0.2059	0.0052	Curcumin
Turmeric sample	0.384	0.2106	0.0052	Curcumin
Turmeric sample	0.384	0.2552	0.0073	Curcumin
Turmeric sample	0.385	0.2595	0.0074	Curcumin
Turmeric sample	0.381	0.2754	0.0087	Curcumin
Turmeric sample	0.381	0.2824	0.0089	Curcumin
Turmeric sample	0.382	0.2993	0.0100	Curcumin
Turmeric sample	0.385	0.3025	0.0100	Curcumin

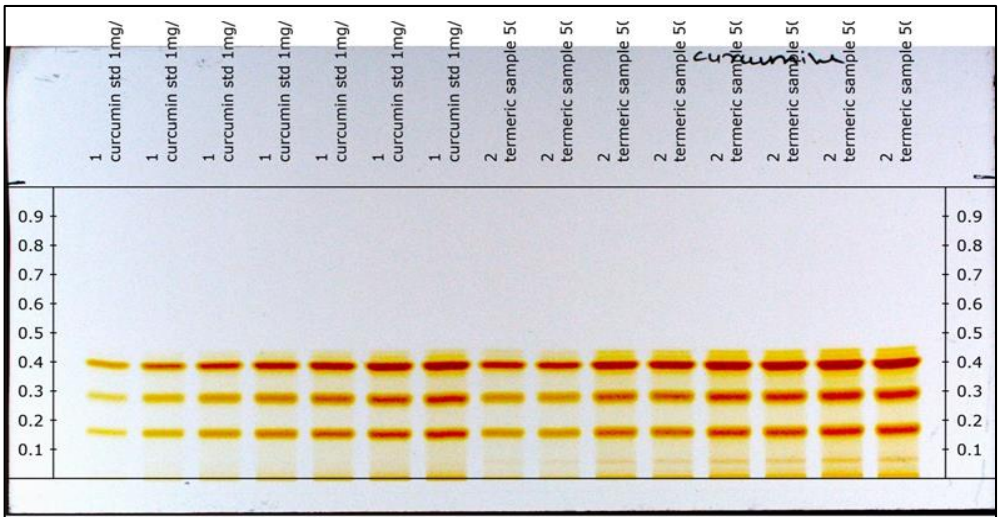

Fig 1: HPTLC Plate developed under visible remission (540nm) showing bands of standards and samples

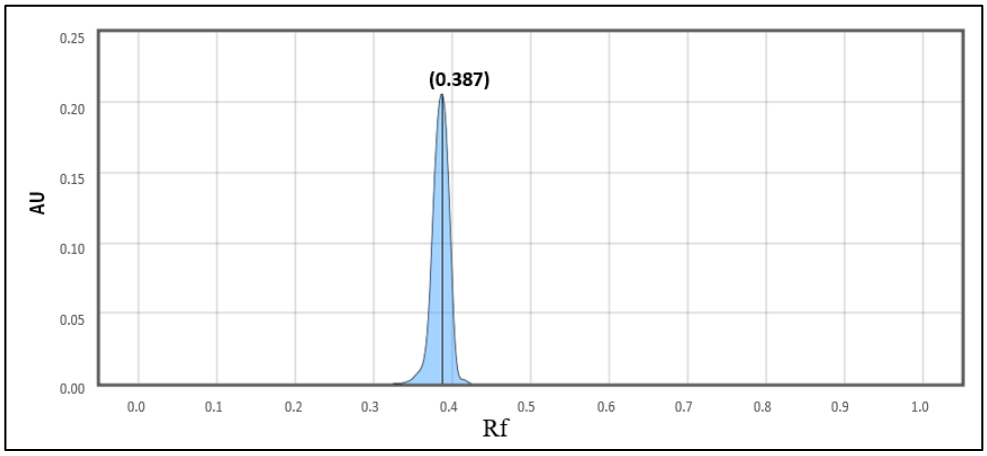

Fig 2: HPTLC plate track peak for identification of curcumin in turmeric sample

For quantification purpose, calibration curve was plotted with five reference samples and four turmeric samples in the CAMAG scanner. Coefficient variation (CV) was 2.49% and correlation coefficient was 99.308583%. Based on the calibration curve, curcumin present in the turmeric sample was evaluated to be 1.631 mg in 50 mg of turmeric sample. The calibration curve was linear within a range of 1.631mg/ml of turmeric sample. Fig.3 showed the calibration curve for relative quantification of curcumin in the turmeric sample.

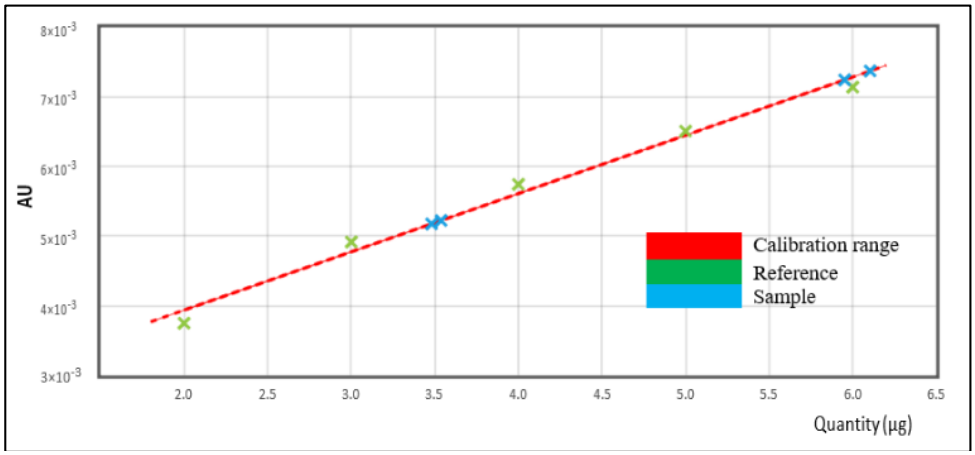

Fig 3: HPTLC calibration curve for evaluation of curcumin in turmeric sample (50mg/ml)

Conclusion

The results obtained in the present study suggest that the roots of *Curcuma longa* cultivated at herbal garden of Ethno Veterinary Herbal Product Research and Development Centre, Veterinary College and Research Institute, Orathanadu contain curcumin, which has potential for use in the health care of animal kingdom.

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