to the cotyledonary leaves. Callus initiation and its further growth was maximum on MS medium supplemented with either kinetin (2 mg/l) or benzylaminopurine (BAP, 2 mg/l) and a low concentration of naphthaleneacetic acid (NAA). Kinetin and NAA at higher concentration caused little or no callus formation. NAA alone could support moderate callus growth only.

Winthin 2–3 weeks, the callus started differentiating into nodular structures and formed shoot buds, which subsequently gave rise to multiple shoots. Frequency of cotyledons responding to plant regeneration depended upon the plant genotype and the hormonal constitution of the medium (table 1). MS medium supplemented with BAP and NAA was most effective and gave highest frequency of shoot regeneration. The frequency of shoot regeneration declined when BAP was substituted with kinetin. Media containing kinetin and NAA together at high concentration or NAA alone gave poor regeneration frequencies.

When 10–15-day-old regenerated shoots were separated and subcultured individually onto fresh medium, multiple shoots (1–25) could be seen regenerating from the callus formed at the base of the transferred shoot. In one case as many as 98 multiple shoots could be obtained from a single cotyledon explant in two subcultures.

There were significant differences in the shoot regeneration response of the three cultivars of *E. sativa*. The best regeneration was observed in cv. T-27, which gave an average of 9.8% explant cultures with shoots. TMH-46 and TMH-48 gave 7% and 5.9% explant cultures with shoots respectively.

Most of the regenerated shoots developed roots in the parent medium. The remaining shoots could be easily rooted when cultured on MS medium supplemented with NAA, after giving a cut at the base.

The study showed that genotype of the plant and hormonal constitution of the medium determine the shoot regeneration response of explant tissue in *E. sativa*. A proper balance of cytokinin and auxin is necessary to achieve high regeneration frequencies. The procedure and the mode of plant regeneration are similar to that reported for other *Brassica* species[6,7]. By using such techniques it may be possible to select genetically modified *E. sativa* through *in vitro* culture and to develop agronomically improved strains of this crop.

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THIN-LAYER CHROMATOGRAPHY ON CELLULOSE

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THIN-LAYER chromatography (TLC)[1,2] is a rapid, simple and versatile method for separation of mixtures of phytochemicals because of its speed and sensitivity as well as the availability of a variety of adsorbents like silica gel, alumina, celite, calcium hydroxide, magnesium phosphate, polyamide, Sephadex, polyvinylpyrrolidone and cellulose, or a mixture of two or more of them.

Ready-to-use TLC plates are not yet common in Indian laboratories, especially plates coated with silica gel, cellulose and polyamide. The preparation of TLC plates coated with silica gel[3] is comparatively easy but their preservation is difficult; on the other hand the preparation of cellulose plates[4] suited for a specific purpose is difficult though the prepared plates are comparatively easily preserved and handled. Under these circumstances and in view of the fact that cellulose TLC shows much higher resolution and speed than PC, it was decided to develop and test cellulose plates for qualitative and preparative analysis. The present study relates to the preparation of cellulose TLC plates and examination

Table 1 *Cellulose TLC of plant constituents*

Compounds	Solvent composition	Detection
Phenol and phenolic acids	Benzene–methanol–water (45:8:4)	Vanillin–conc. HCl
Phenylpropanoids	Aceticacid (15%) Benzene–aceticacid–water (6:7:3) Water (15%)	UV/UV–ammonia
Flavones and flavonols	Aceticacid conc. HCl acetic-acid–water (3:30:10) Phenol (water-saturated) Water	UV/UV–ammonia
	n-Butanol–aceticacid–water (3:1:1)	UV/diphenylborinate
Anthocyanins	Amyl alcohol–aceticacid–water (2:1:1) Formicacid–conc. HCl–water (10:1:3)	Visible/UV/UV–ammonia
Chalcones and aurones	Isopropanol–acetone–water (5:1:4) *n*-Propanol–aceticacid–water (1:1:1)	Visible/UV/UV–ammonia
Sugars (monosaccharides)	Benzene–*n*-butanol–pyridine–water (5:1:3:3) *n*-Butanol–aceticacid–water (4:1:5, upper) *n*-Butanol–ethanol–water (4:1:5) Phenol	Aniline–hydrogen–phthalate

of their suitability for the separation of phytochemicals.

Two types of cellulose adsorbents, viz. microcrystalline cellulose for TLC (Loba-Chemie Indoaustranal Co., Bombay, India) (A), and cellulose powder adsorbent for TLC (Biochemicals Unit, University of Delhi, India) (B), were used.

The technique of preparing cellulose chromoplates involves the mixing of 20 g of A in 140 ml of water and homogenization in a mechanical mixer for 3–5 min. Coating is done using standard TLC applicators, with film thickness adjusted to 0.5 mm. The plate was gently rocked until the material was evenly distributed over the surface. After air-drying (28–30 C) for about 5 h the plate was ready for use. No binder was needed because of the particulate nature of cellulose powder. Care should be taken to eliminate air bubbles while transferring adsorbent slurry to the applicator. Plates were prepared in the same manner for the cellulose sample B, except that the slurry was prepared by mixing 20 g of adsorbent in 100 ml of water.

Fifteen plates (20 × 5 × 0.05 cm) could be prepared in the case of cellulose powder A and 12 chromoplates in the case of cellulose powder B with the quantities mentioned. For two-dimensional separation, square chromoplates (20 × 20 × 0.05 cm) were found suitable. Of the two samples A and B, A was observed to be superior with regard to compactness of the spot and resolution.

Separation into homogeneous components from mixtures of hydrophilic plant constituents was achieved using cellulose plates made as described and using the developing solvents indicated in table 1.

Preliminary investigations were performed for hydrophilic plant products. Development was accomplished with various solvent mixtures prepared from analytical grade reagents. The most suitable ones for the common plant constituents (especially phenolic compounds and sugars) are given in table 1.

Our study confirmed the general observation of the superiority of cellulose in TLC separation. It has an edge over the seemingly similar technique of silica gel TLC in the following aspects: the plates can be safely handled and preserved, the resolution is superior, and the eluate after centrifugation is generally free from the adsorbent. Mixtures of flavonoids containing up to nine constituents could be separated by a one-stage 2D-TLC over cellulose whereas 2D silica gel TLC as well as 2D-PC fails to achieve this. Details of the compounds isolated and identified from plant extracts will be published elsewhere.

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ESTIMATION OF QUERCETIN IN THE LEAVES OF *MAESA INDICA* WALL BY A NEW COLORIMETRIC METHOD

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THE leaves of *Maesa indica* are used in curries in North India and also as fish poison. Extracts of the branchlets, leaves, bark and stem have insecticidal activity[1]. Locally the plant is called 'vavding'. It is used as a blood purifier and as an anthelminthic by the local people. This communication reports isolation and estimation of quercetin from the leaves *M. indica* from Goa.

The methods reported in the literature for quercetin estimation are Folin–Ciocalteau spectrophotometry[2–5], fluorimetry[6], colorimetry by using Folin–Ciocalteau reagent[7], and by treating with NaOAc, HOAc (3:1) and $AlCl_3$ (ref. 8). In the present work a simple and sensitive method that gives very stable colour for more than one hour is described. In the Folin–Ciocalteau method the authors described the method for general phenolic substances from apple juice, while the present method is specific for quercetin only. In the NaOAc, HOAc and $AlCl_3$ method the colour obtained is stable for 20 min whereas in the present method the colour is stable for more than one hour. The present method is more sensitive than the earlier methods, and is good up to 2 μg quercetin.

Mature leaves of *M. indica* were collected at the beginning of winter from the Morlem forests of Sattari taluka in Goa. A fruiting voucher specimen of the plant is preserved in the phytochemistry research lab., Goa College of Pharmacy. The leaves were washed in running tap-water, air-dried and powdered. The leaf powder (100 g) was defatted with petroleum ether. The marc was then extracted with methanol, and the extract reduced to 50 ml and mixed with 6% HCl (50 ml). The solution was heated on a water bath for 45 min. The hydrolysate was extracted thoroughly with ether in a separating funnel. On separation the ether layer was concentrated to residue[9]. The residue was digested with H_2O, filtered and then dissolved in *n*-propanol. It was further purified by preparative TLC (silica gel G, using EtOAc: HCOOH:AcOH:H_2O, 100:11:11:27)[10,11]. The compound obtained was recrystallized from methanol and was confirmed from UV and IR spectra as quercetin.

A new colorimetric method was developed for quantitative analysis of quercetin by reacting it with acidic ammonium molybdate in *n*-propanol medium using Speckol Spectrocolorimeter (Carl Zeiss Jena).

Quercetin (10 mg) was dissolved in 100 ml of *n*-propanol to obtain a reference solution. 10 g of leaf powder was defatted with petroleum ether and thoroughly extracted with methanol in a continuous hot extractor. The methanolic extract was filtered and concentrated to 5 ml. This concentrate was subjected to acid hydrolysis as in the isolation procedure and the volume was made up to 50 ml. Two ml of this was made up to 100 ml with *n*-propanol to obtain a test solution.

Three activated silica gel G plates were streaked with 0.25, 0.5 and 0.75 ml of the reference and test solutions. The solvent system used was using EtOAc:HCOOH:AcOH:H_2O (100:11:11:27). Scrapings of the band corresponding to quercetin were transferred to a stoppered test tube and extracted vigorously with 5 ml of *n*-propanol. After centrifuging the supernatant liquid was filtered through Whatman No. 42 filter paper into a 10 ml volumetric flask. To the above filtrate, 0.5 ml of 0.1 N H_2SO_4 and 1 ml of 10% (w/v) aqueous ammonium molybdate were added. The resulting yellow chromogen was measured at 420 nm. A graph of absorbance versus concentration was plotted and was found to be linear over the concentration range 2 to 14 μg/ml, indicating conformity with Beer's law.

The quercetin content of the leaves of *M. indica* was found to be 3.03%. Quercetin has also been reported in *Maesa macrophylla*[12].