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Research Article
HPTLC Analysis of Stem Bark Extracts of *Terminalia chebula* Retz. for Alkaloid Profile

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Abstract
Terminalia chebula Retz. commonly known as Haritaki is a deciduous tree having extraordinary powers of healing with a wide spectrum of biological activity. The aim of the present study was to check the presence of phytochemical constituent alkaloid, using HPTLC analysis. The stem bark extracts of *Terminalia chebula* was prepared using different solvents like methanol, chloroform and petroleum ether. The extracts have been subjected to HPTLC analysis for alkaloid profile. Colchicine was used as standard. The presence of alkaloid in all the three solvent extract samples were confirmed based on the colour zones obtained. The presence of alkaloid was indicated by bright orange and brown coloured zones under daylight mode. High performance thin-layer chromatography analysis is a powerful technique and thereby significantly increased precision and high throughput. Hence this profile analysis would pave the way for the future phytoconstituents detection and herbal formulation.

Key words: *Terminalia chebula*, stem bark, HPTLC, alkaloid, herbal formulation, haritaki, phytochemicals, throughput

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. In India it is estimated that about 6000 plants are in use in traditional, folk and herbal medicine. Thus upto 80% of population depend directly on the traditional medicine for the primarily health care (Kirby, 1996). There are very few medicinal plants of commercial importance which are not found in India. In recent years secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). The use of herbal drugs is once more escalating in the form of Complementary and Alternative Medicine (CAM) (Cooper and Blais, 2004). This phenomenon has been mirrored by an increasing attention to phytomedicines as a form of alternative therapy by the health professions. Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities (Dev, 1999).

Nature has been a rich source of medicinal plants and an impressive number of modern drug have been isolated from natural source. Medicinal plants used by traditional people are proving to be an important resource of potentially therapeutic drugs (Cox and Balick, 1994). The therapeutic actions of the plants are due to the various chemical compounds mainly secondary metabolites, present in them. The phytochemicals have been used as drugs, dyes and food additives etc. These phytochemical studies are not limited to isolation of active principle but towards new frontiers dealing with plant physiology, ecology, agriculture, medicine etc. (Cai *et al*., 2004).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases (Argal and Pathak, 2006). Plants have the major advantage of still being the cheapest and most effective alternative source of drugs (Van der Watt and Pretorius, 2001). The search for newer source of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). Plants are the cheapest and safer alternative source of antimicrobials (Van der Watt and Pretorius, 2001; Sharif and Banik, 2006; Doughari *et al*., 2007).

Terminalia chebula is a flowering evergreen plant species belonging to the family Combretaceae. It is a medium to large deciduous tree attaining a height of upto 30 m with widely spreading branches and a broad roundish crown. *Terminalia chebula* has been reported to exhibit a variety of biological activities including anticancer (Saleem *et al*., 2002), antidiabetic (Sabu and Kuttan, 2002) and antiviral properties (Ahn *et al*., 2002). The stem bark of *Terminalia chebula* was found to possess antioxidant activity (Elias *et al*., 2011) and antimicrobial activity (Varkey and Kasthuri, 2012).

HPTLC has become a routine analytical technique for herbal drug standardization due to its advantages of low operating cost, high sample throughput, speed, simplicity and need for minimum sample clean up, reproducible, reliable, accurate and robust (Nyiredy, 2001; Sherma and Fried, 1996). So the stem bark extract of *Terminalia chebula* were subjected to phytochemical analysis for the identification of bioactive phytocompounds.

MATERIALS AND METHODS

Collection of the plant: The stem barks of *Terminalia chebula* were collected from Nilgiri district, Tamil Nadu, India. This study was conducted from November, 2013 until April, 2014.

Solvents extract preparation: The plant materials were washed and dried in an oven at 45°C. Powdered samples were successively extracted in a soxhlet apparatus using low, medium and high polar solvents such as petroleum ether (40-60°C), chloroform (59.5-61.5°C) and methanol (64-65.5°C) for 16-18 h. The extract was concentrated and stored in refrigerator (4°C).

HPTLC analysis of plant extracts for alkaloid profile: The HPTLC fingerprinting analysis, 2 µL of the test solution and 2 µL of standard solution were loaded as 5 mm band length in the 3 and 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase (Alkaloid) and the plate was developed in the respective mobile phase upto 90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254 nm and UV 366 nm. The developed plate was sprayed with respective spray reagent (flavonoids and phenols) and dried at 100°C in hot air oven. The plate was photo documented at daylight and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber. After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 500 nm. The peak table, peak display and peak densitogram were noted (Kumudhavalli *et al*., 2010; Wagner and Bladt, 1996).

Test solution preparation: The given dried extract 100 mg was weighed in an electronic balance (Afcoset) accurately and dissolved in 1 mL of appropriate solvent based on the extracted solvent and centrifuged at 3000 rpm for 5 min. These solutions were used as test solution for HPTLC analysis.

Sample application: Two microliter of test solutions and 2 µL of standard solution were loaded as 6 mm band length in the 5×10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument.

Spot development: The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase (Alkaloid) and the plate was developed in the respective mobile phase upto 90 mm.

Photo-documentation: The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at white light, UV 254 nm and UV 366 nm.

Derivatization: The developed plate was sprayed with respective spray reagent (Alkaloid) and dried at 100°C in hot air oven. The plate was photo-documented in day light and UV 366 nm mode using photo-documentation (CAMAG REPROSTAR 3) chamber.

Scanning: Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV 254 nm. Ethyl acetate-methanol-water (100:13.5:10) was used as mobile phase and Dragendorff's reagent followed by 10% ethanolic sulphuric acid reagent as spray reagent. The peak table, peak display and peak densitogram were noted.

RESULTS

HPTLC analysis was carried out for the three different solvent extracts of *Terminalia chebula* to investigate its alkaloid profile. Colchicine was used as a reference compound to compare the alkaloid compound present in methanol, chloroform and petroleum ether extracted samples. Analysis of samples before and after derivatization under daylight and UV light at two different wavelengths (366 and 254 nm) was presented in Fig. 1 and 2. All the Rf values obtained during this analysis was presented in Table 1. Baseline and densitogram display of colchicine was used to identify the Rf values of all the test components after scanning at 254 nm (Fig. 3a and b). The presence of alkaloid in all the three solvent extract samples were confirmed based on the colour zones obtained. The presence of alkaloid was indicated by bright orange and brown coloured zones under daylight mode. Following were the results obtained for the three extracts of *Terminalia chebula* bark powder.

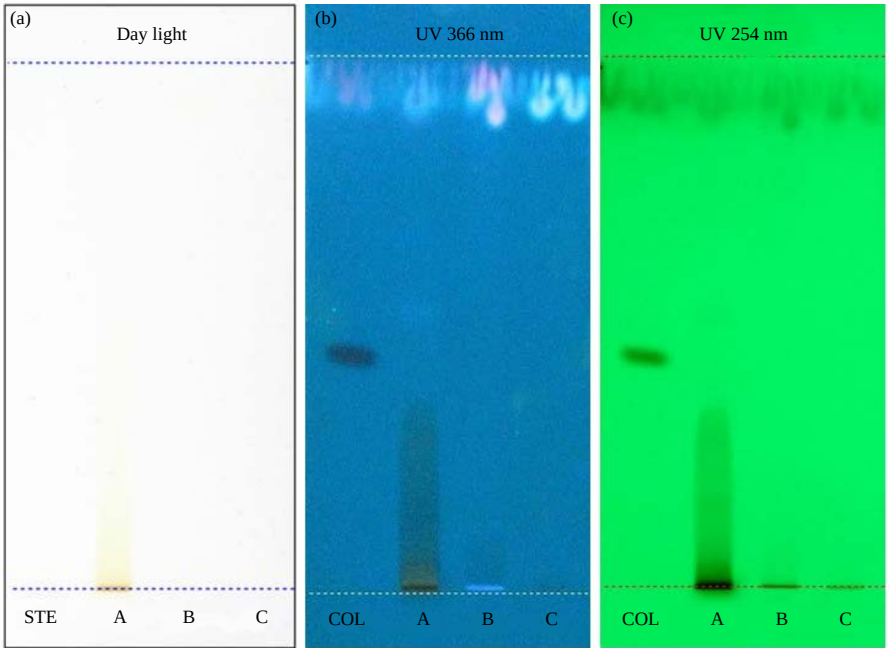

Fig. 1(a-c): Chromatogram before derivatization, (a) Day light, (b) UV 366 and (c) UV 254 nm

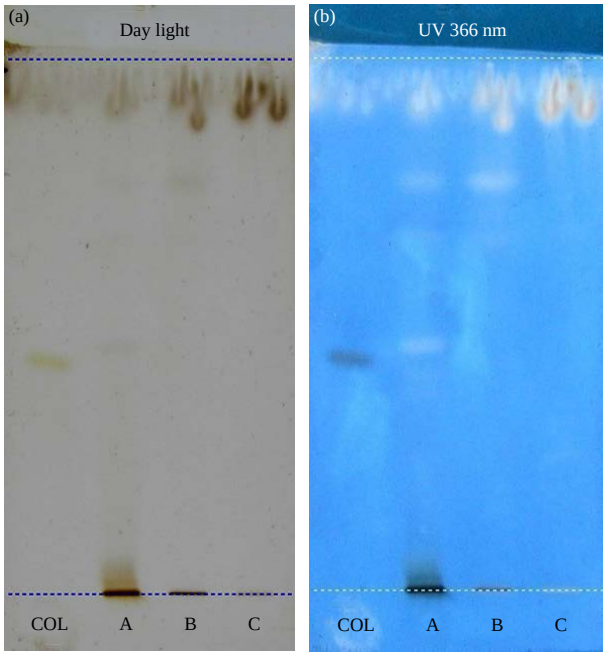

Fig. 2(a-b): Chromatogram after derivatization, (a) Day light and (b) UV 366 nm

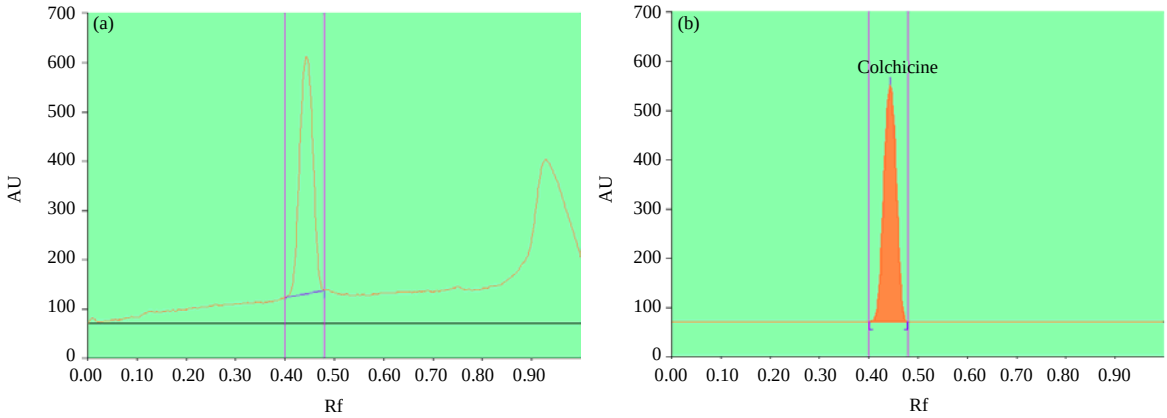

Fig. 3(a-b): Track COL-colchicine standard (a) Baseline and (b) Peak densitogram display (Scanned at 254 nm)

Table 1: HPTLC analysis of bark extracts for alkaloid profile

Tracks	Peak	Rf	Height	Area	Assigned substance
COL	1	0.44	503.1	14161.8	Colchicine standard
Sample A	1	0.01	46.9	200.2	Unknown
Sample A	2	0.17	47.8	1799.9	Unknown
Sample A	3	0.29	121.8	10651.0	Unknown
Sample A	4	0.53	42.0	1157.4	Alkaloid 1
Sample A	5	0.76	4.1	57.8	Alkaloid 2
Sample A	6	0.92	179.0	10991.8	Unknown
Sample B	1	0.07	11.9	297.4	Unknown
Sample B	2	0.76	11.1	201.4	Alkaloid 1
Sample B	2	0.76	11.1	201.4	Alkaloid 1
Sample B	3	0.93	171.3	12063.8	Unknown

HPTLC of methanolic extract: HPTLC finger print scanned at wavelength 254 nm for methanolic bark extract of *Terminalia chebula* showed six polyvalent phytoconstituents with their corresponding Rf values of 0.01, 0.17, 0.29, 0.53, 0.76 and 0.92. All the Rf values were presented in baseline and densitogram display scanned at 254 nm (Fig. 4a and b). From the analysis it was found that the component number 4 and 5 was confirmed as alkaloid compounds 1 and 2 after comparing with the zone colour respective to the reference compound colchicine (Table 1). Bright orange and brown colour zones were observed under daylight mode for the samples and reference colchicine.

HPTLC of chloroform extract: Chloroform bark extract of *Terminalia chebula* showed three polyvalent

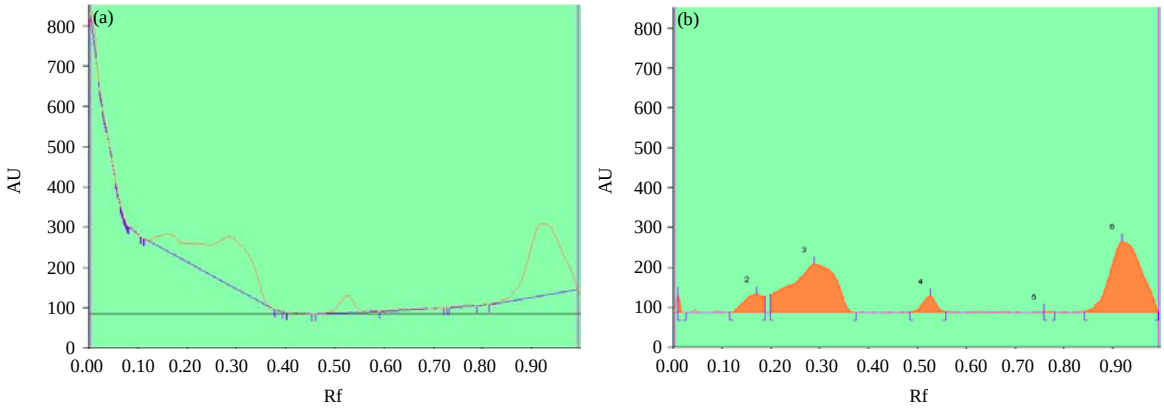

Fig. 4(a-b): Track A-sample A methanolic extract (a) Baseline and (b) Peak densitogram display (Scanned at 254 nm)

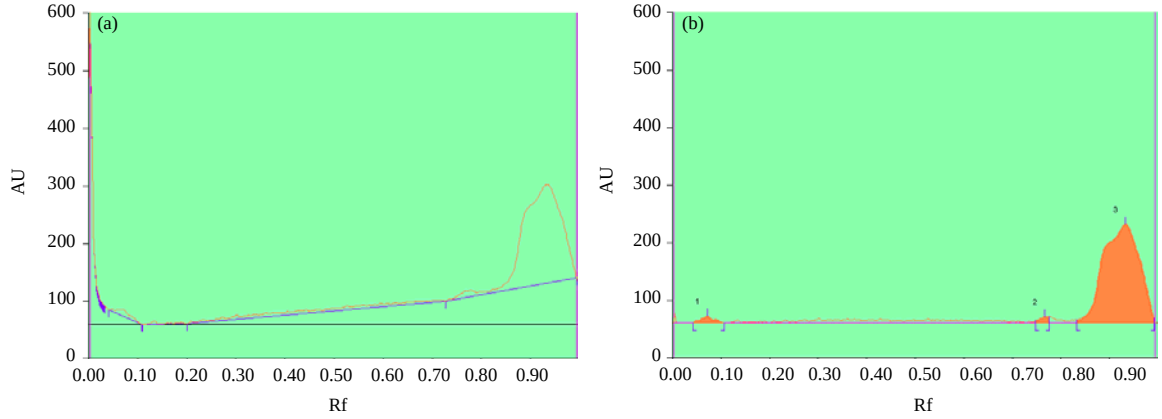

Fig. 5(a-b): Track B-sample B chloroform extract (a) Baseline and (b) Peak densitogram display (Scanned at 254 nm)

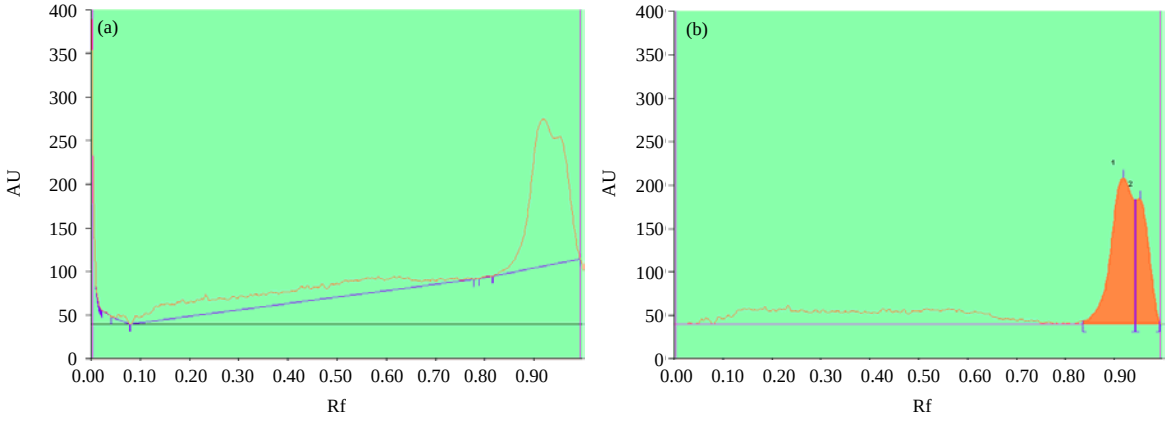

Fig. 6(a-b): Track C-sample C petroleum ether extract (a) Baseline and (b) Peak densitogram display (Scanned at 254 nm)

phytoconstituents with their corresponding Rf values of 0.92, 0.07 and 0.76 (Table 1, Fig. 5a and b). From the analysis it was found that the colour of the zone corresponding to the component number 3 and that of reference compound colchicine was very similar.

HPTLC of petroleum ether extract: In contrast, the petroleum ether extracts does not contain any alkaloid compounds when compared to the zone colour of the reference compound (Fig. 6a and b). The 3 D display of all the three samples compared with the reference colchicine compound scanned

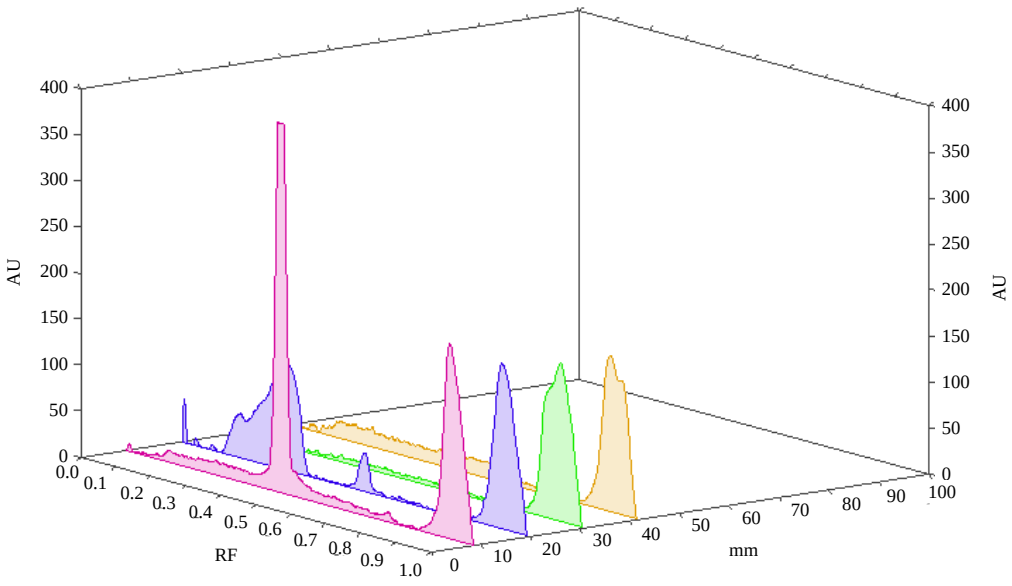

Fig. 7: 3 D display of all tracks

at 254 nm was also presented in Fig. 7. The display presented the variations in the peak heights of all the three samples. Colchicine showed the maximum peak height of 503.1 (Table 1) followed by sixth component in sample. A (methanolic extract-179.0), third component of sample-B (chloroform extract-171.3) and first component of sample-C (petroleum ether-168.9).

DISCUSSION

When the findings were compared to the research works of Tambe *et al*. (2013) and Devi *et al*. (2013) it has been found that the researchers during their preliminary phytochemical screening of bark extracts of *Symplocos racemosa* and *Ficus nervosa*, respectively investigated and extrapolated the presence of alkaloids, triterpenes, tannins, saponins, glycosides, phenolic compounds and flavonoids. From this analysis it was revealed that all types of plant bark extracts may contain similar phytochemical constituents. Similarly, HPTLC analysis of *Hydnocarpus macrocarpa* showed the presence of alkaloids, flavonoids, glycosides, tannins and saponins. The diversity and richness of phytochemical compounds makes it a source for further phytochemical and pharmacological investigation (David and George, 2014).

In the present study the phytochemical constituent were identified and confirmed based on the colour zone obtained during the HPTLC analysis. The confirmation was done after comparing the colour of the zone with the colour of the reference compounds under daylight and UV light after derivatization in the chromatogram. Also further confirmation was carried out based on their densitometric analysis. This was found supportive when similar approach was handled by Sasikumar *et al*. (2009). In their report, they identified blue and brown colour (zone) for the methanol extract of *Pandanus odoratissimus* under UV light after derivatization in the chromatogram. Also they confirmed the presence of polyphenols among the five peaks based on the densitometric HPTLC analysis. Similarly, coloured zone of bright orange representing the presence of alkaloids were identified in the present study.

High performance thin layer chromatography profile studies on the alkaloids of *Albizia lebbeck* observed various alkaloids profile. Similar to the observations, in the present study we produced the HPTLC profile for methanol, chloroform and petroleum ether and it was revealed that secondary metabolites are produced by a large variety of plants, of which 10-20% are alkaloids and are part of group natural products (Bobby *et al*., 2012). A combined method of high performance liquid chromatograph-electrospray-ionization mass spectrometer (HPLC-ESI-MS/MS) coupled with a photodiode array detector (HPLC-DAD) was applied to the qualitative and quantitative analyses of alkaloids in *Cortex phellodendri*. This method proved to be a useful tool in quality control of herbal medicines which can be performed for the present study in the near future (Zhu *et al*., 2011).

CONCLUSION

The utility of the drug is due to the chemical which they contain. Sometime, these drugs contain several constituents which are inert therapeutically. Therefore, we should know about the active and inactive constituents of the crude drug. The preliminary phytochemical screening is a qualitative chemical evaluation which indicates spectrum of chemical constituents present in a plant drug. The HPTLC analysis of stem bark extract of *Terminalia chebula* was accurate, specific and precise. This detailed chemical profile may be useful in the identification as well as quality evaluation of drugs with respect to plants. It may also enable a particular plant to be identified and distinguished from closely related species. Hence, the profile analysis gives an insight into the evaluation of desired marketed products.

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