

TABLE 1: VALUES OF EXPONENTIAL CONSTANT, N AND APPARENT VISCOSITY OF THE GUAR GUM SOLUTIONS

Concentration (% w/v)	Exponential constant (N)	Apparent viscosity (η')
1	1.4077	122.4052
0.8	1.2242	16.47404
0.6	1.0607	2.591194

function; whereas, a first order relation was found to exist between apparent viscosity and concentration of guar gum (1.0% w/v, 0.8% w/v and 0.6% w/v), complying with the equation $\eta = e^{Kc+b}$, where the values of the constants K and b obtained were 9.638 and -4.857, respectively.

GG showed a non-Newtonian pseudoplastic rheological behaviour at all the pH's studied. Fig. 3 shows the effect of pH on viscosity of the GG solutions at different shear rates. It can be seen that from pH 1.5 to pH

10.5, the viscosity remains nearly constant at a given shear rate. At pH 12, the viscosity of the GG solution decreases. Thus, solutions of guar gum exhibited non-Newtonian, pseudoplastic behaviour at all pHs. The viscosity of the solutions was found to remain fairly constant from pH 1.5 to 10.5.

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Spectrophotometric Estimation of Total Alkaloids from *Cinchona officinalis* Stem Bark and Marketed Formulations Containing *Cinchona*

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The alkaloids of stem bark of *Cinchona officinalis* and the marketed formulations containing the stem bark of *Cinchona* were estimated by spectrophotometric method using tropaeolin'OO' for the formation of colour complex. In *Cinchona* stem bark, the alkaloids are bound to tannins. The method adopted for the extraction of alkaloids from the samples has the advantage of extraction of mainly the alkaloids and not the other interfering substances like tannins. The method of analysis was found to be sensitive, precise and accurate and it can be adopted for routine quality control purposes.

Cinchona species (Family: Rubiaceae) are known for antimalarial activity and other potential therapeutic values of the stem bark. Stem bark of *Cinchona* species is included in many herbal, Ayurvedic and Homeopathic

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formulations. The active principles are quinoline alkaloids, the important ones being quinine, quinidine, cinchonine and cinchonidine. Quinine and its analogues are mainly used as antimalarials for the treatment of *Plasmodium falciparum* infections. Quinidine is known for its antiarrhythmic properties¹. In the present paper, the total

alkaloids from *C. officinalis* stem bark and the marketed formulations containing *Cinchona* were estimated by spectrophotometric method using tropaeolin 'OO'. This method has applicability in the routine quality control for the estimation of total alkaloids from both the crude drug *Cinchona* stem bark and its formulations.

Quinine reference standard was obtained as a gift from Centre for Bio-Pharmaceutical Sciences, Leiden University, The Netherlands. Samples of *C. officinalis* stem bark were obtained from three different parts of the country (A-Ooty, B-Ahmedabad and C-Mumbai) and were authenticated in our Pharmacognosy and Phytochemistry Department and voucher specimens were preserved. Three different brands (H-1, H-2, H-3) of Homeopathic mother tinctures (China - Q) and one herbal tablet formulation (*Cinchona* tablet, Gujarat Ayurved Vikas Mandal, Junagad, Gujarat) containing *Cinchona* stem bark were purchased from the local market. All the chemicals used were of analytical grade. Reagents include acetate buffer of pH 4.6 (5.4 g of sodium acetate and 2.4 g of glacial acetic acid in 100 ml of double distilled water), saturated solution of tropaeolin 'OO' and acid reagent (1 ml of concentrated sulphuric acid + 99 ml of methanol).

For the linearity curve preparation, standard solution of quinine (100 mg/ml) was prepared in methanol, aliquots of 1-3.5 ml were taken and colourimetric analysis was carried out following the method of Haussler². In brief, the method is as follows: Solvent was removed at room temperature from the above aliquots and 5 ml of acetate buffer and 3 ml of tropaeolin 'OO' solution were added. The complex thus formed was extracted with chloroform (3 x 15 ml). The chloroform extract was transferred to a 50 ml volumetric flask containing 3 ml of acid reagent and the volume was made up with chloroform. The violet chromogen developed was measured at 545 nm against blank, using Double beam UV-VIS Spectrophotometer (Jasco-7850). Linearity curve of concentration vs absorbance was plotted.

For the extraction of alkaloids from the samples (1 g each of stem bark powder/1 ml each of Homeopathic mother tinctures/1 g tablet powder (from 20 tablets)), method given in European Pharmacopoeia³ was adopted, with certain modifications where 20% sodium hydroxide solution was replaced with 25% ammonia solution and tragacanth was replaced with carboxy methyl cellulose (sodium salt). Suitable quantity of sample solutions was taken and colour was developed as described above. Absorbance of coloured solution was recorded at 545 nm.

The amount of total alkaloids from sample solutions were calculated from the calibration curve and represented as quinine.

The proposed method is based on the formation of charge transfer complex between alkaloids and tropaeolin 'OO' at pH 4.6 which can be extracted either in chloroform or dichloromethane, followed by its reaction with acid reagent, to give violet coloured chromogen with λ_{max} of 545 nm⁴⁻⁸. Various parameters involved in colour development such as effect of pH, amount of acetate buffer, amount of tropaeolin 'OO' solution, amount of acid reagent and time involved at various stages of reaction were optimized. Maximum colour intensity was obtained with 3 ml of saturated solution of tropaeolin 'OO', 5 ml of acetate buffer and 3 ml of acid reagent. The violet coloured chromogen was stable up to 3 h. In *Cinchona*, the alkaloids are present in combination with quinic acid and cinchotannic acid^{9,10}. Tannins interfere during the extraction of the alkaloids by the conventional acid-base extraction method. In the present experiment, during the extraction of alkaloids from the stem bark as well as the formulations, carboxy methyl cellulose (sodium salt) was used to adsorb the interfering substances. This facilitated the efficient extraction of the alkaloids in the organic solvents subsequently.

The method of analysis was validated for precision and accuracy. Linearity range, slope, intercept and correlation co-efficient are given in Table 1. The method was checked for precision by repeating the experiment 5 times with the same quantity of quinine. Percentage relative standard deviation was found to be 1.06. The accuracy of the method was determined by performing the recovery study at two levels by adding known amounts of the drug to the sample. The average percentage recovery was found to be 99.77 (Table 2). The method was applied for the estimation of total alkaloids from *C. officinalis*

TABLE 1: METHOD VALIDATION PARAMETERS

Parameters	Results
Linearity range ($\mu\text{g/ml}$)	2 - 7
Correlation coefficient (r)	0.999
Slope	0.162
Intercept	- 0.036
Precision (n = 5; % RSD)	\pm 1.06
Accuracy (%)	99.77

TABLE 2: PERCENT RECOVERY STUDIES

Amount of total alkaloid present (µg)	Amount of quinine added (µg)	Amount of total alkaloid found (µg)*	% recovery*	Average % recovery
100	100	199.4 ± 3.97	99.7 ± 1.98	99.8
100	200	299.6 ± 3.13	99.9 ± 1.04	

*Each value is the mean and standard deviation of three determinations

stem bark from three different regions, one herbal tablet preparation and three Homeopathic formulations (Table 3). In *Cinchona* stem bark the amount of alkaloids varies widely (4 to 7.5%)¹¹. In the present investigation, we found that the samples from Ooty and Mumbai had about 6.9 and 5.08 % of total alkaloids respectively, while the sample from Ahmedabad was of poor quality with only 1.1% of total alkaloids. The alkaloidal contents of Homeopathic samples were found to vary from 0.65 to 1 mg/ml, while the herbal tablet contained 3.7% (w/w) total alkaloids. The method for estimation of total alkaloids developed in the present investigation is sensitive, efficient, precise and accurate and may be used for routine

estimation of total alkaloids from *Cinchona* stem bark and its formulations.

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TABLE 3: ESTIMATION OF TOTAL ALKALOIDS OF *C. OFFICINALIS*

Sample	Total alkaloids*
Stem bark	(% w/w)
A	6.91 ± 0.132
B	1.14 ± 0.020
C	5.10 ± 0.113
Formulations :	
Homeopathic samples**	(mg/ml)
H-1	0.65 ± 0.033
H-2	1.02 ± 0.013
H-3	0.85 ± 0.013
II. Herbal tablet	(% w/w)
	3.78 ± 0.140

A, B and C are *Cinchona* stem bark samples obtained from Ooty, Ahmedabad and Bombay, respectively. *denotes that each value is a mean ± standard deviation of 3 determinations. ** Indicate that H-1, H-2 and H-3 are three different brands of Homeopathic mother tinctures containing *Cinchona*.