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PHARMACOGNOSTICAL STANDARDIZATION AND CHROMATOGRAPHIC EVALUATION OF *TECOMELLA UNDULATA* STEM BARK

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ABSTRACT

Tecomella undulata (Family Bignoniaceae), commonly known as honey tree, ammora, rohida, desert teak or white cedar is widely used as an ethno medicine in India. Its stems have found extensive usage by some ethnic communities in reliving body pain and in treating snake bite. However, detailed scientific information is not available to identify the plant material and to ascertain its quality and purity. Therefore in this background meticulous pharmacognostical and chromatographic study of stem bark has been carried to establish the pharmacognostical standards and phytochemical evaluation. Physico-chemical parameters were studied for systemic identification and authentification of leaves. A qualitative fingerprinting of *Tecomella undulata* (TU), extracts have been performed by HPLC methods. Transverse Sections of stem bark shows presence of various characteristic features like single layer thick walled epidermis, some of its cell forming non- glandular and glandular hairs. In physico-chemical evaluation the water soluble ash 4.02±0.21% w/w is higher than acid insoluble ash 1.49±0.08% w/w. Alcohol & water soluble extractive value was found to be 8.97±0.93 and 9.31±0.54% w/w respectively. Elemental analysis shows presence of high amount of iron (618 ppm) as compare to other elements. Successive extractive value of methanolic extract 7.05±0.12 % w/w is higher than the other extractive values. TLC and HPLC chromatogram shows the presence of various chemical

constituents in different R_f value and retention time, which provide quality evaluation and standardization of crude drug. This study provides referential information for identification and standardization of *Tecomella undulata* stem bark and its extracts.

Keywords: *Tecomella undulata*.; stem; Qualitative Chromatographic evaluation; Histological parameters; Physico-chemical evaluations; elemental analysis

Abbreviations: TU- *Tecomella undulata* Linn; FAA - formalin: acetic acid: 70 % ethyl alcohol; HPLC-High Performance Liquid chromatography; PE-Pet ether extract ; TU-Toluene extract; CF-Chloroform extract; EA-Ethyl acetate extract; ME-Methanol extract.

INTRODUCTION

Traditional herbal medicines and their preparations have been widely used for the thousands of years in developing and developed countries owing to its natural origin and lesser side effects or dissatisfaction with the results of synthetic drug [1]. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. Analytical methods such as photometric analysis (UV, IR, MS, and NMR), thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations [2]. Majority of crude herbs come from nature like wild and it is collected to evaluate quality parameters by which presence of various phytochemicals can be confirmed. Due to heterogenous composition in form of whole plant natural product standardisation is not a simple task. Authenticaton, pharmacognostic evaluation, phytochemical analysis and sensitive chromatographic separation techniques (HPLC) provide a forefront for development of rapid and cost effective methods for standardization and characterization of herbal drugs and to unknot novel phytoconstituents [3, 4, 5].

Tecomella undulata (family Bignoniaceae), commonly known as Ammora (in English) or Rohida and is locally known as honey tree, desert teak, marwar teak or white cedar, is widely distributed in Arabia, southern Pakistan and North west India upto an elevation of 1200 meters. In Pakistan it is found in Baluchistan and Sind [6]. Due to the presence of secondary metabolites with therapeutic potential *Tecomella undulata* gained importance in medicinal world. As a result, significant number of articles has been published traversing over the second half of the 20^{th} century. Phytochemical studies of

plant parts has revealed presence of pharmacologically relevant compounds such as Iridoid glycosides [7], naphthaquinone [8, 9] phytosterols, flavanoid glycosides, flavonol [10], fatty alcohol [11], fatty acids [12] and triterpenoids [13]. *Tecomella undulata* is well known for its medicinal value in Ayurveda [14, 15] and traditionally, it is extensively used for treatment of several diseases like liver, spleen, internal tumours, diseases of abdomen, wound healing, conjunctivitis, hepatosplenomegaly, blood purifier, syphilis, gonorrhoea and rewarding in hepatitis [6]. Various reported Pharmacological activity of *Tecomella undulata* is anti-HIV [16], Antibacterial activity [17], Antimicrobial [18], analgesic and anti-inflammatory [19], antioxidants [20] and hepatoprotective [6]. In spite of numerous medicinal uses of TU, standardization parameters for its stem bark have not been reported. Hence, the present investigation is an attempt in this direction to evaluate morphological, microscopic and physico-chemical characters along with phytochemical screening fluorescence analysis of powdered crude drug and HPLC analysis of its extracts.

MATERIAL AND METHODS

Plant Material

T. undulata stem bark was collected in the month of September 2013 from the campus of The M. S. University of Baroda, Vadodara, India. Samples were authenticated in the Botany Department and were deposited in the Pharmacy Department of The M. S. University of Baroda.

Chemicals and instruments

All the solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (95%), n-butanol and reagents viz. formalin, acetic acid, ethyl alcohol, tertiary-butyl alcohol, paraffin wax, safranin, fast-green, potassium iodide were of analytical grade and were procured from E. Merck, India. HPLC (Shimadzu, Kyoto, Japan) and HPTLC (Camag, Switzerland) are the major instruments used for the fingerprinting studies. Microscopic photographs were taken using a Magnus MIPS-4613 microscope attached with Magnus live USB 2.0 camera.

Macroscopic and Microscopic analysis

Morphological features of the stem bark were examined after the collection. Fresh root bark were collected from the plant and fixed in FAA (formalin: acetic acid: 70% ethyl alcohol) for the anatomical studies. Twenty four hours later, the samples were dehydrated with a graded series of tertiary-butyl alcohol (TBA). Infiltration of the samples was carried out by gradual addition of paraffin wax (melting point 58º – 60º C) until TBA solution attained super saturation. The sample

was then successively embedded into paraffin blocks.

Sectioning

With the aid of an MC 930 advanced precision rotary microtome the paraffin-embedded samples were sectioned. Serial sections were cut at thickness of 10 - 12 µm. After that dewaxing of the sections was performed by treating the specimen slides sequentially with xylol, xylol + alcohol, water and finally, with staining fluid. Wherever necessary, sections were also stained with fast-green, safranin and iodine in potassium iodide in order to evaluate cambium, phloem, crystals and medullary rays. The Section was examined and by using Leica research microscope using ×40 objective the representative areas were photographed. The micro powder analysis was done according to the method [21, 22]

Physico-chemical analysis

Physicochemical analysis i.e., percentage ash values and extractive values were determined according to the official methods described [23] and the WHO guidelines for the quality control methods for medicinal plant materials [24]. Fluorescence analysis was carried out according to the method [25]. For estimation of heavy metals about 5 gm of powdered drug material was ignited in muffle furnace to obtain total ash. 100 mg of the ash was dissolved in 10 ml of 1 N HCl and then the solution was filtered and diluted to 50 ml with distilled water and used for quantitative determination of heavy metals by the atomic absorption spectrophotometer (AAS) (SYSTRONIC 128), coupled with hydride generator and hollow cathode lamps for different elements including heavy metals.

Preliminary phytochemical screening

Preliminary phytochemical tests for the presence of alkaloids, sugars, phenols, flavanoids, saponins, steroids, tannins, coumarins, terpenoids and glycosides were performed on the petroleum ether, chloroform, methanol and aqueous extracts of TU leaves by using standard procedures described by Harborne, 1998 [26].

HPLC studies

The HPLC analysis of methanolic extract was carried with the chromatographic system (Shimadzu, Kyoto, Japan) consisted of a Shimadzu LC-20 AT Prominence solvent delivery module, a manual Rheodyne injector (Perkin Elmer, Mumbai, India) with a 20 mL fixed loop, and an SPD-20A Prominence (Shimadzu) UV-Vis detector. The separation was performed on a Hypersil C18 column (particle size 5 mm; 250 ´ 4.6 mm id; Thermoquest, Cheshire, UK) preceded by an ODS (Thermoquest) guard column (10 mm, 10 ´ 5 mm id) at an ambient temperature.

Chromatographic data were recorded and processed using a Spinchrom Chromatographic Station® CFR Version 2.4.0.193 (Spinchrom Pvt. Ltd, Chennai, India). Peak purity analysis was carried out using an SPD M20A photo-diode array (PDA) detector from Shimadzu. The mobile phase consisted of Acetonitrile-water (75: 25 v/v) and the separation was performed by using isocratic elution at a flow rate of 1 ml/ min. The samples were run for 40 minutes. Detection was done at 254, 366 nm by UV detector.

Sample preparation

TU stem bark (5 g coarse powder) was extracted separately twice, with methanol (2 x 45 ml) under reflux (45 min each time) on a water bath. The final extract was taken to dryness in a rotary evaporator and 10 mg of the extract was transferred to a 10 mL volumetric flask and the volume was made up with HPLC grade methanol.

RESULTS AND DISCUSSION

Macroscopic characters

Stem bark of *Tecomella undulata*, occur in flat or slightly curved pieces, about 6-8 mm in thickness. The outer surface of bark is greyish brown with occasional small dark patches. Longitudinal zigzag furrows and irregular transverse cracks are present on outer surface making it rough. A few vertically elongated lenticels are also present. The inner surface of bark is smooth and buff to brownish in colour. Photographs of stem bark is given in Figure 1 and there characteristic features are reported in **Table 1**.

Microscopic characters

Microscopically young stem shows a single layer thick walled epidermis, some of its cell forming non- glandular and glandular hairs, glandular hairs called stalk and 12-16 celled head, and multiseriate and branched non-glandular hairs. The microscopic features of stem bark T.S. are depicted in **Figure 2**.

Powder characters

The stem bark powder is greenish brown in color with a characteristic odor. The powder microscopy shows some important features like unicellular glandular trichomes, periderm cells and scleraids/ scleratic cells. These features are depicted in **Figure 3**.

Physicochemical studies

Ash values of *Tecomella undulata* stem bark shows relatively high total ash indicating high quantity of carbonates and oxide. A low acid insoluble ash values are obtained in stem bark of *Tecomella undulata* indicates less siliceous material like earth or sand.

Extractive values determining the amount of active constituents extracted with

solvents from a given amount of medicinal plant material. They are useful for evaluation of crude drugs and give in idea about the chemical nature of chemical constituents present in them. The water soluble extractive values of *Tecomella undulata* stem bark are high as compared to alcohol soluble extractive values, indicating that a high quantity of polar constituents are present in them as compared to non polar constituents. These results are depicted in **Table 2**.

Elemental analysis

All the living organisms require inorganic elements for their growth and survival. Medicinal plant contain considerable amounts of mineral constituents, in particular, the presence of essential elements (Mg, Mn, Zn and many others) is a prerequisite for correct growth and development of plants. Inorganic elements in plants also plays role in the accumulation of secondary metabolites such as alkaloids, glycosides, terpenoids, phenolic compounds etc. as their responsible for the activity of a number of enzymatic systems, which in turn regulate the metabolic pathways leading to the synthesis of these compounds. Results of elemental analysis of plant material under study viz., stem bark are given in **Table 3**.

Successive solvent extraction

Percentage yield and colour of the selected successive extracts are recorded in **Table 4**. The successive solvent extraction of the drug with solvents of increasing polarity results in the separation of the constituents according their polarity. Stem bark of *Tecomella undulata* gives maximum extractive value with methanol where as with ethyl acetate these extractive values were found to be very less.

Phytochemical analysis of successive extracts

Phytochemical investigation revealed the presence of alkaloids, phenols/tannins, flavanoids, saponins, steroids & glycosides while terpenoids found absent. Results of phytochemical analysis for leaves and stem bark of all the extracts are summarized in **Table 5**.

TLC Studies

For total terpenoids (triterpenes) TLC studies were done (toluene- chloroform-ethanol, 4: 4: 1; AS reagent) on the successive extract of stem bark and results revealed that toluene and ethyl acetate, positive results for the presence of

terpenoids. Detection of flavonoids using (ethyl acetate – formic acid– acetic acid– water; 100: 11:11: 27; Natural product reagent/PEG) showed 2, 1 and 2 light and dark blue florescent spots in chloroform, ethyl acetate and methanol extracts respectively. Determination of alkaloids using toluene– ethyl acetate– diethyl amine; 70: 20: 10; Dragendorff reagent showed 1, 2 and 2 orange colour spots in chloroform, ethyl acetate and methanol extracts respectively. The results obtained from TLC of the successive extracts are recorded in **Table 6**.

HPLC Studies

Results of HPLC analysis of *Tecomella undulata* stem bark methanolic extract (mobile phase, methanol-water; 1:1, flow rate; 2ml/min, detection; 254nm) shows that various constituents are present in it and their peaks are found in the chromatogram at different retention time (in mins) such as 0.963, 2.447, 2.820, 3.107, 3.440, 4.470, 4.870, 5.177, 5.507, 5.853, 6.237, 6.650, 6.833. 7.740, 8.457, 8.703, 11.480, 12.373, 13.533, 14.487, 15.357, 15.717, 16.823, 18.177 and 21.210. Similarly, At 366 nm the stem bark methanolic extract shows various peaks at retention time (in mins) 2.440, 2.790, 3.447, 4.277, 5.173, 6.257, 6.633, 6.950, 7.753, 9.103 and 13.603 mins. HPLC chromatograms of stem bark are shown in **Figure 4 and 5**.

Figure 1: Photographs of stem bark of *Tecomella undulate*

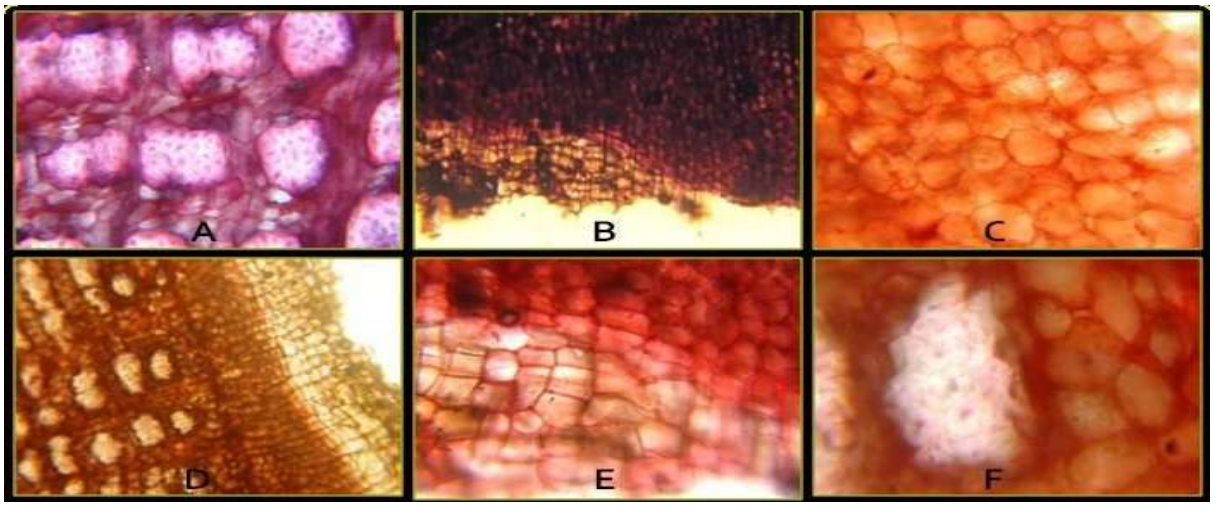

Figure 2 Characteristic features of transverse section of *Tecomella undulata* stem bark. A) Layers of sclerotic cells alternating with phloem layers; B) Periderm with low magnification; C) Cells of phelloderm; D) T.S. of bark showing periderm and outer pheloem; E) Periderm 6 layers (Phellum); F) Phloem layer and scleratic cells patch.

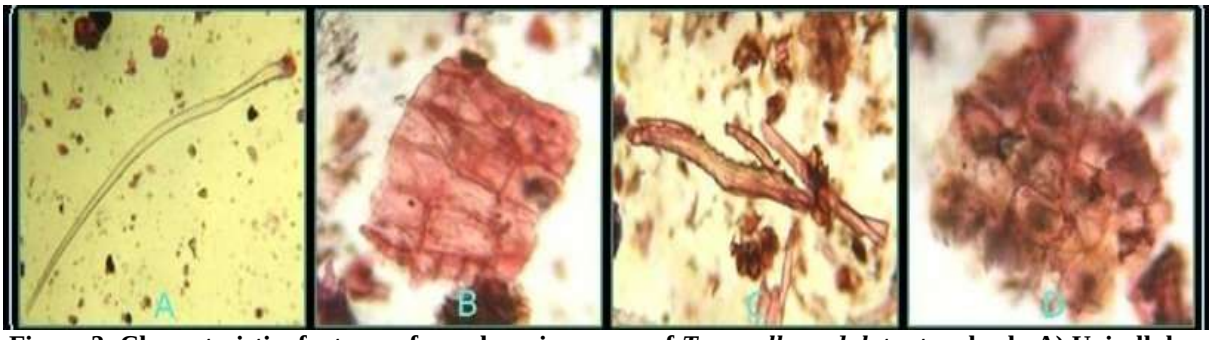

Figure 3: Characteristics features of powder microscopy of *Tecomella undulata* stem bark. A) Unicellular glandular trichomes, B) Periderm cells, C) Sclereids/scleratic cells, D) Periderm cells

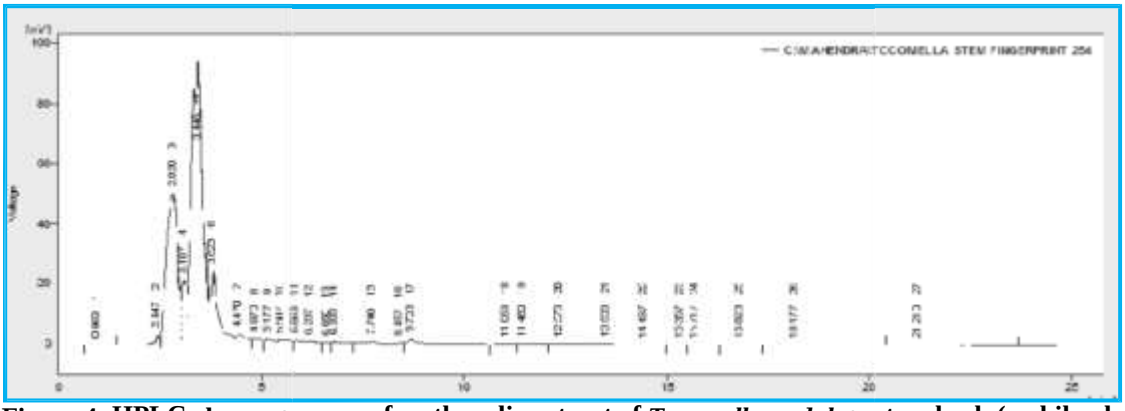

Figure 4: HPLC chromatogram of methanolic extract of *Tecomella undulata* stem bark (mobile phase, acetonitrile-water; 75:25, flow rate; 1ml/min, detection; UV detector at 254nm)

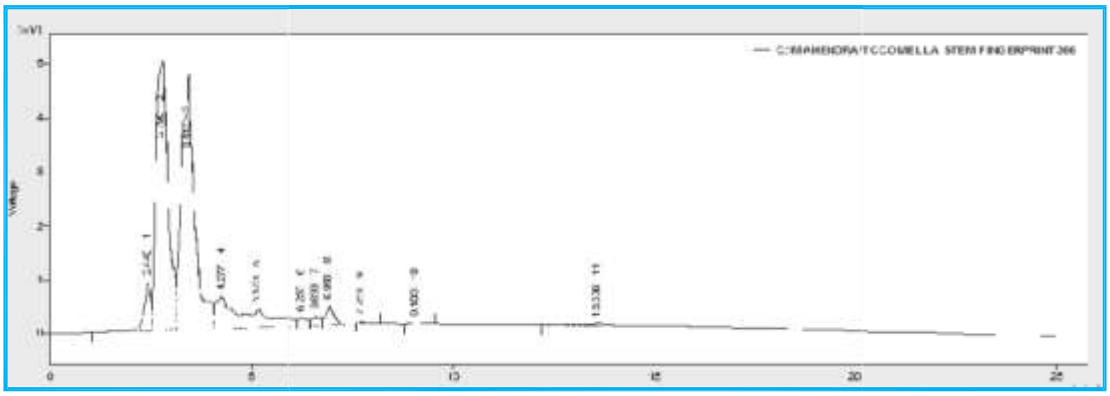

Figure 5: HPLC chromatogram of methanolic extract of *Tecomella undulata* stem bark (mobile phase, Acetonitrile-water; 75: 25, flow rate; 1ml/min, detection; UV detector at 366 nm)

Table 1: Characteristic macroscopic features of *Tecomella undulata* stem bark

Serial number	Features	Observation of stem bark
1	Colour -Inner surface -Outer surface	 Dark brownish Dull brown grey or grey in colour
2	Odour	Odourless
3	Taste	Tasteless
4	Shape	Curved
5	Fracture	Short
6	Size -Length -Breadth	 20 cm 8 mm (thickness)

Table 2 Physicochemical analysis of *Tecomella undulata* stems bark

Parameters	Values% (w/w) * ±SEM
	Stem bark
Total Ash	7.92±0.33
Acid insoluble ash	1.49±0.08
Water soluble ash	4.02±0.21
Water soluble extractive values	9.31±0.54
Alcohol soluble extractive values	8.97±0.93

***Values expressed as mean of three readings**

Table 3 Elemental analysis values of *Tecomella undulata* stem bark

Elements	Values in ppm/ %
	Stem bark
Potassium	0.45 %
Sodium	0.05 %
Iron	618.00 ppm
Zinc	10.00 ppm
Copper	5.8 ppm
Mangenese	30.00 ppm

Table 4: Successive extractive values of *Tecomella undulata* stem bark

Solvent	Values%(w/w)*	
	Stem bark	Appearance
Petroleum ether	0.88	Yellowish
Benzene	1.78	Greenish yellow
Chloroform	2.53	Brownish
Ethyl acetate	0.43	Brownish
Methanol	7.05	Brownish
Water/Aqueous	6.05	Brown

Table 5 Preliminary phytochemical investigations of *Tecomella undulata* stem bark

Phytochemicals	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	-	+	+	-
Phenols / tannins	+	+	+	+
Flavanoids	-	-	+	+
Saponins	-	+	+	+
Terpenoids	-	-	-	-
Glycosides	-	-	+	+

'SB' Stem bark; '+' presence; '-' absence

Table 6: TLC Studies on successive extracts of stem bark *Tecomella undulata*

Constituents	Pet-ether (Rf)	Toluene (Rf)	Chloroform (Rf)	Ethyl acetate (Rf)	Methanol (Rf)
Alkaloids	Not detected	Not detected	0.33	0.35, 0.66	0.18, 0.63
Flavanoids and Phenolics	Not detected	Not detected	0.39, 0.84	0.82	0.76, 0.82
Terpenoids	Not detected	0.14, 0.17, 0.24, 0.41, 0.26, 0.53	Not detected	Not detected	Not detected

CONCLUSIONS

The present study of HPLC fingerprinting on the extracts from stem bark of *Tecomella undulata* will provide useful information for its identification. Morphological, microscopic and physicochemical standards discussed herein can be considered as identifying parameters to substantiate and authenticate the drug.

REFERENCES

[1] WHO 2001. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines. p. 1

[2] Booksh, K.S, Kowalski B. R., 1994. Theory of analytical chemistry, Anal. Chem. 66, 782A-791A.

[3] Jain, M., Kapadia, R., Jadeja, R.N., Thounaojam, M.C., Devkar, R.V., Mishra, S.H., 2012. Hepatoprotective potential of *Tecomella undulata* stem bark is partially due to the presence of betulinic acid. J Ethnopharmacol. **143**, 194-200.

[4] Jain, M., Trivedi, A., Mishra, S.H., 2010. TLC Determination of marmesin, a biologically active marker from *Feronia limonia* L. American J. Plant Sci. **1**, 12-16.

[5] Jain, M., Kapadia, R., Albert, S., Mishra, S.H., 2011. Standardization of *Feronia limonia* L. leaves by HPLC, HPTLC, physico-chemical and histological parameters. Bol Latinoam Caribe Plant Med Aromat. 10, 525-35.

[6] Chal, J., Kumar, V., Kaushik, S., 2011. A Phytopharmacological overview on *Tecomella undulata* G. Don. J Appl Pharmaceut Sci. 1, 11-12

[7] Singh, P., Khandelwal, P., Hara, N., Asai, T., Fujimoto, Y., 2008. Radermachol and Naphthoquinone derivatives from *Tecomella undulata*: Complete 1H and 13 C NMR assignments of radermachol with the aid of computational 13C shift prediction. Indian J Chem. 47B, 1865-1870.

[8] Verma, K.S., Jain, A.K., Gupta, S.R., 1986. Structure of undulatin: New iridoid glycosides from *Tecomella undulata*. Planta Med. 52, 359- 362.

[9] Joshi, K.C., Singh, P., Pardasani, R.T., 1977. Quinones and other constituents from the roots of *Tecomella undulata*. Planta Med. 31, 14-16.

[10] Taneja, S.C., Bhatnagar, R.P., Jiwari, H.P., 1975. Chemical

constituents of flowers of *Tecomella undulata*. Indian J Chem. 13, 427-428

[11] Randhawa, G.S., Mukhopadhyay, A., 2004. Floriculture in India. Allied Publication (p) Ltd: Mumbai. 225-226

[12] Khare, C.P., 2004. Indian Herbal Remedies, Rational western therapy, Ayurvedic and other traditional usage. Botany. Society of new age herbals: New Delhi (India). 67.

[13] Mukerji, A.K., 1977. Triacontanol and triterpenes from *Tecomella undulate*. Xi world forestry congress. 3, 1.

[14] Anonymus 2003. Genetic diversity analysis in *Tecomella undulata*. The Biome News. 4, 8-9.

[15] Oudhia, P., 2005. Medicinal herbs of Chhattisgarh, India having less known traditional uses 72 Roheda (*Tecomella undulata* family Bignoniaceae). Available from: http://botanical. com/site/column_poudhia/articles/449_roheda.html

[16] Azam, M.M., 1999. Anti-HIV agents and other compounds from *Tecomella undulata*. Orient J Chem. 15, 375-377.

[17] Gehlot, D., Bohra, A., 2000. Antibacterial effect of some leaf extracts on *Salmonella typhi*. Indian J Med Sci. 54, 102-105.

[18] Parekh, J., Chanda, S.C., 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J Biol. 31, 53-58.

[19] Ahmad, F., Khan, R.A., Rasheed, S., 1994. Preliminary screening of methanolic extracts of Celastrus peniculatus and *Tecomella undulata* for analgesic and anti inflammatory activities. J Ethnopharmacol. 42, 193-198.

[20] Gautam, S.P., Ganjiwale, R.O., Yeole, P.G., 2007. Free radical scavenging properties of aqueous extract of Tecomella undulata leaves. Indian J Nat Prod. 23, 33-37.

[21] Brain, K.R., Turner, T.D., 1975. The Practical Evaluation of Phytopharmaceuticals, Wright-Scientechnica, Bristol.144.

[22] Kokate, C.K., 1986. Practical Pharmacognosy, 1st ed., Vallabh Prakashan, New Delhi.

[23] Indian Pharmacopoeia, 1996. Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi. 4, 53-54.

[24] WHO/PHARM/92.559/rev.1., 1992. Quality Control Methods for Medicinal Plant Materials, Organisation Mondiale De La Sante, Geneva. 9, 22 - 34.

[25] Chase, C.R., Pratt, R.J., 1949. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharmacol Assoc**.** 38, 324 - 33.

[26] Harbome, J.B., 1998. Methods of extraction and isolation. In: Phytochemical Methods, Chapman & Hall, London.