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SEASONAL EFFECTS ON SOME PHYTOCHEMICALS ACCUMULATION OF *TECOMELLA UNDULATA* (SM.) SEEM.

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ABSTRACT:

Tecomella undulata (Sm.) Seem. is an important agro-forestry tree belongs to family Bignoniaceae, commonly known as "Desert teak" or "Rohiro" found in the western parts of India, which has been included in the list of endangered plant species due to over exploitation. Phytochemical analysis showed the presence of important classes of phytoconstituents like flavonoids, tannins, alkaloids were studied in different extracts, which are helpful for curing the various diseases like liver and spleen diseases, tumors, syphilis, gonorrhea. It possesses significant anticancer activity, hepatoprotective, anti HIV, analgesic activity and antibacterial activity. The quantitative accumulation of the phytochemicals may be different during different seasons and the rate of accumulation in different plant parts may be affected due to environmental changes during seasonal variation. The result of the study can be useful for the selection of the season and plant parts to get maximum quantity of the secondary metabolites.

Keywords: *Tecomella undulata* (Sm.) Seem, Secondary metabolite, Phytochemical, Quantitative estimation.

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INTRODUCTION

Tecomella undulata (Sm.) Seem (Bignoniaceae) is a deciduous or nearly evergreen tree of desert or dry regions of India. It is also known as Rugtrora (Hindi) and Ragatrohida (Gujarati). Leaves are narrow, somewhat lance shaped with wavy margins, 5-12 cm long. In spring time it produces beautiful showy tubular flowers in yellow, orange and red colors. The plant is useful for planting in shelterbelt plantations and may become an important agro-forestry tree in arid and semi-arid areas for the production of high quality timber, in addition to fuel wood and fodder. It possesses significant anticancer activity, hepatoprotective, analgesic activity and antibacterial activity[1, 2, 3]. The bark of the young branches possesses mild relaxant, cardio tonic activities and also used in the treatment of syphilis and eczema[4]. The bark contains tecomin, alkenes, alkanols, β-sitosterols, chromone glycosides, undulatoside, A and B, iridoidglucosides, tecomelloside, tecoside, lapachol, veratric acid etc [5]. Seasonal effects on bioactive compounds were studied in Aconitum spp from Manipur, India[6] and in Calotropis procera (Ait). R. Br.[7].

Phytochemical compounds of the Tecomella plant is not explored yet and it's not found reported in any scientific literature. So, the present study can be useful to get maximum quantify the phytochemicals of the plant for better knowledge of pharmaceutical constitution of plant.

MATERIALS AND METHODS

Collection and Authentication of Plant: Healthy and disease free plant parts like leaves and stems of *Tecomella* was collected in three different seasons winter, summer and monsoon seasons from botanical garden of the Department of Life Sciences at Hemchandracharya North Gujarat University, Patan. Plant authentication was done by the Department of Life Sciences including botany at Hemchandracharya North Gujarat University, Patan.

Preparation of Plant Extracts: 10 gm of collected leaves and stems were used after air drying and all the

samples were ground in mechanical grinder and powdered material was kept in an airtight container for further use. Hot extraction of plant samples was prepared by using Soxhlet apparatus. The advantage of this system is that instead of many portion of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds. In *Tecomella,* methanol was used as a solvent. In preparation of extraction, methanol was taken in similar volume (150 ml)[8].

Qualitative Analysis: The extracts were subjected to phytochemical test for the detection of organic constituents which include alkaloids, flavonoids, steroids, saponins and tannins according to standard protocol[9].

Quantitative Analysis: Quantitative estimation of total phenol and total flavonoids was determined by standard protocol. Methanol and acetone extracts were used for Quantitative estimation.

A) Spot Test for Alkaloids

a) Mayer's Reagent: 1.36g $HgCl_2$ was dissolved was dissolved in 60 ml distilled water and 5 g KI in 10 ml DW. Both solutions were mixed and diluted up to 100ml with DW. White colour precipitates indicate the alkaloids.

b) Wagner' Reagent: 1.27 g I_2 was dissolved in 2 g KI and diluted up to 100 ml with DW. White Brown flocculent colour Precipitates, which shows the presence of alkaloids.

c) Dragenddroff's Reagent: 8 g Bismuth nitrate was dissolved in 20 ml HNO_3 and 27.2 g KI in 50 ml DW. Both solution were mixed and allowed to stand before use. Brown colour Precipitates, which shows the presence of alkaloids.

d) Scheibler's Reagent: 100 g Sodium tungstate and 70 g dibasic sodium phosphate (Na_2HPO_4) were dissolved in 500 ml DW and it was acidified with Nitric acid. White amorphous colour Precipitates indicate the presence of alkaloids.

f) Ammonium Reineckate's reagent: It was 4% HCl solution. Pink fluorescent colour Precipitates indicate the presence of alkaloids.

g) Folin–Ciocalteau's phenol Reagent: Commercially available reagent (2N) was diluted with an equal amount of DW. White Precipitates indicate the presence of alkaloids.

B) Spot Test for Flavonoids

a) Magnesium Ribbon Test: 1 g dried powered plant material was taken in a test tube and covered with 10 ml 95% ethanol. The test tube containing mixture was kept in boiling water bath for 15 minutes. The extract was filtered and small piece of magnesium ribbon was added. 3 drops of concentrated HCl was added. The occurrence of a red colour indicated the presence of flavonoids.

C) Spot Test for Steroids:

2 g dry sample was taken. 15 ml petroleum ether was added to it and it was incubated for 12–24 hrs at room temperature. It was filtered and the filtrate to dried. To the dry filtrate 2-3 drops of acetic anhydride was added and 2-3 drops of concentrated H_2SO_4. Presence of pink/blue colour indicates the presence of steroids.

D) Spot Test Analysis for Saponins

a) **Red Test:** 2 gm dry sample was taken. 15 ml chloroform was added to it and it was incubated for 12-24 hrs at room temperature. It was filtered and the filtrate to dried. To the dry filtrate add 2-3 drops of concentrated H_2SO_4.Presence of blue or blue green or reddish brown or presence of pink coloured ring indicated the presence of saponin.
b) **Honey Comb Froth:** 2 gm dry sample was taken. 80% 15 ml ethanol was added to it and it was incubated for 12-24 hrs at room temperature. It was filtered and the filtrate to dried. To the dry filtrate 5 ml D W was added. It was shaken vigorously. The formation of honey comb like froth indicated the presence of saponin.

E) Spot Test Analysis for Tannins

a) **Gelatin Test:** 5 gm dry sample was taken. To it 50 ml water was added and the whole mixture was boiled for 30-45 minutes. It was filtered and to that filtrate and to that filtrate 2-3 drops of 2% gelatin was added. To it acidified NaCl was added drop wise. White colour Precipitates shows the presence of tannins.
b) **Lead acetate Test:** 2 gm dry sample was taken. 80% 15 ml ethanol was added to it and it was incubated for 12-24 hrs at room temperature. It was filtered and the filtrate to dried. To the dry filtrate add 2ml of D.W and 5% lead acetate. White colour Precipitates shows the presence of tannins.

Total Phenol Determination: Take 100 mg of plant materials and add 10ml 80% methanol then centrifuged at 5000-10,000 g for 10 minutes after it collect the supernant- I and repeat same for the residue and collect supernant- II. Then mixed the supernant- I and supernant- II and used as plant sample aliquot. Total phenolic Content of the extracts was determined by Folin ciocalteu reagent method[10]. Plant extract (1ml) was mixed with Ciocalteu reagent (0.1ml, 1N) and allow standing for 15 min. Then 5 ml of saturated Na_2CO_3 was added. The mixtures were allowed to stand for 30 min

at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Tannic acid was used as a standard. Total phenol values are expressed in terms of tannic acid equivalent (mg/ml of extracted compound). Calculation for flavonoids was done using the regression formula and the result was expressed as mg phenol/ ml Plant material. All the values were mean of five replicates and it was represented in graph with ±S.D.

Total Flavonoids Determination: Take 10 g air dried plant material and different calluses, add 100 ml of petroleum ether then kept on a rotary shaker at 190-220 rpm for 24h.supernant was discarded and put the residue at room temperature for petroleum ether was evaporated from the powder. In the powder add 100ml of methanol then kept on rotary shaker at 190-220 rpm for 24h. The extract was centrifuged at 5000g for 10 minutes and supernatant use as plant sample[11]. Aluminum chloride spectrophotometrically method[12] was used to determine flavonoids content. Plant extract (1ml) in methanol was mixed with 1ml methanol, 0.5 aluminum chloride (1.2%) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; then the absorbance was measured at 415nm. Rutin was used as standard. Flavonoids content is expressed in terms of rutin equivalent (mg/ml of extracted compound). Calculation for flavonoids was done using the regression formula. The result was expressed as mg Flavonoids/ml Plant material. All the values were mean of five replicates and it was represented in graph with ±S.D.

RESULTS AND DISCUSSION

Seasonal variation is pronounced in different phytochemicals content of a taxon investigated. The preliminary qualitative phytochemical screening of the crude powder of *Tecomella* was done to assess the presence of bioactive components. The successive extracts of stems and leaves of *Tecomella* have revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins (Table 1). Standard curve of tannic acid for total phenol and standard curve of rutin for flavonoids were used to quantitative estimation (Fig. 1 and Fig. 3).

In summer season total phenol and total flavonoids content were 31.04 mg/ml and 16 mg/ml in leaves respectively and it shows higher content then other two seasons (fig. 2 and fig. 4). Same things observe in stem, in summer season both value was higher in compare to two other season. Total phenol and total flavonoids content was higher in methanol extracts than acetone extracts and that was 14.77±0.08 mg g^{-1} in acetone and 34.14±0.16 mg g^{-1} in methanol extract for leaves of *Tecomella*[13]. Different plants have different phytochemicals accumulation rate during different seasons like total phenol and total flavonoids was higher in summer seasons for *Tecomella* but the phenol content was higher in winter in *Calotropis.* In *Calotropis procera* (Ait.) R. Br tannin content was higher in monsoon for all the parts except for stem showed higher in summer season[7] but in *Juniperus* species extractable components increase toward the end of the growing season, reaching a maximum during the winter and declining in the spring for reveled different and foliage was extremely rich among all the plant parts[14]. For the plants secondary metabolites accumulation rate was different during season because of various environmental factors.

Table. 1: Seasonal Wise Qualitative Analysis of Phytochemicals of *Tecomella* Plant Parts.

S. NO	Name of phytochemicals	Name of Test	Season wise leaf samples			Season wise stem samples		
			SUM	MON	WIN	SUM	MON	WIN
1	Alkaloids	Mayer	++	+++	+++	+	-	-
		Wagner	+++	+++	+++	++	+	+
		Dragendorff	+	+	++	+++	+++	+++
		Scheibler	+++	++	+	+	-	-
		Ammonium Reineckate	++	++	+++	+++	+++	++
		Folin-ciocalteau	++	++	++	+	+	-
2	Flavonoids	Magnesium ribbon	+++	++	++	+	-	-
3	Steroid		+++	++	++	++	+	+
4	Saponins	Ring test	+++	++	+++	+	-	+
		Honey comb froth	+++	++	+++	+++	+	+++
5	Tannin	Gelatin	++	+ +	++	+++	+	+++
		Lead acetate	++	-	+ +	+++	+	+ + +

Note: + = less, ++ = medium, +++ = high,-=absent, sum= summer, mon =monsoon, win=winter

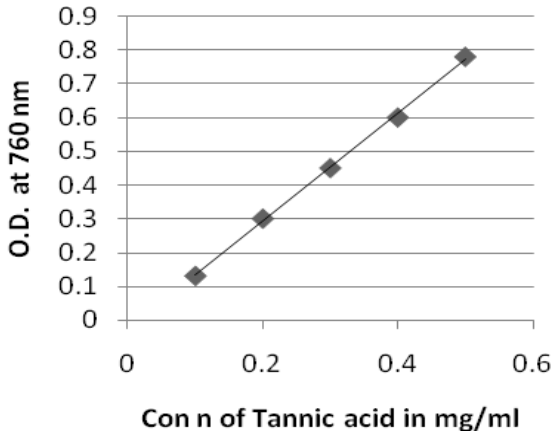

Fig. 1: Std. curve of Tannic acid for totalphenols

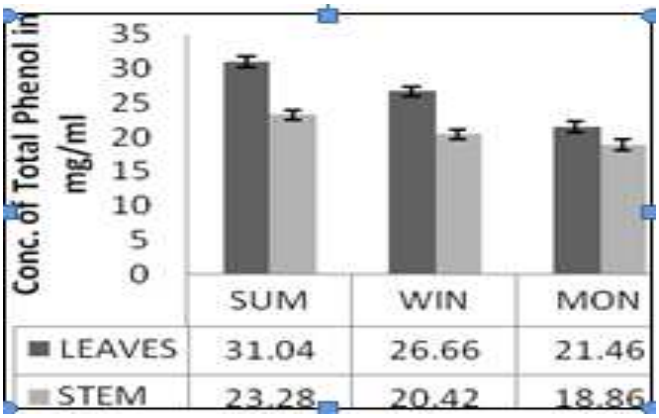

Fig. 2: Total Phenol in Different Seasons of T*ecomella* Plant parts

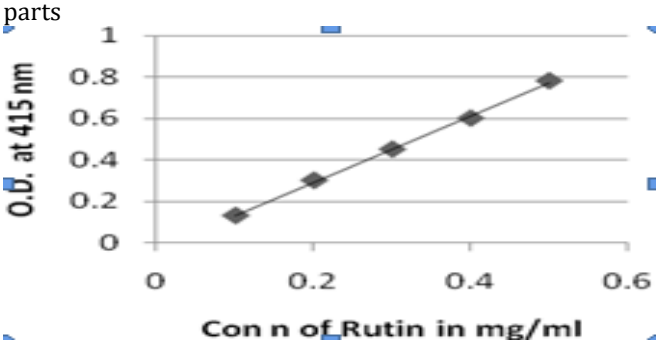

Fig. 3: Standard Curve of Rutin for Total flavonoids

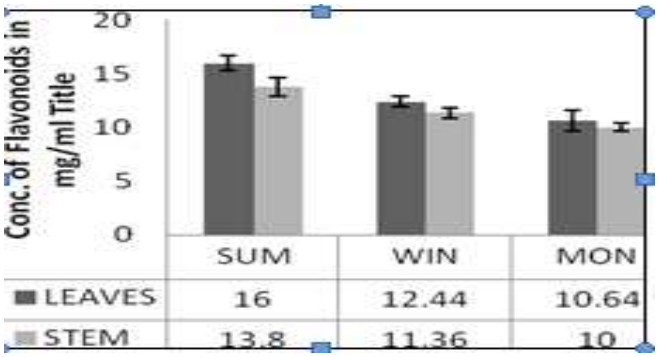

Fig. 4: Total flavonoids in Different Seasons of *Tecomella* Plant parts

CONCLUSION

The valuable medicinal plant *Tecomella undulata* (Sm.) Seem shows variation in phytochemical accumulation during different season. The qualitative screening shows presence of alkaloids, flavonoids, steroids, saponins and tannin all in leaf during all three seasons but in stem it was more in summer season in compare to other two seasons. Quantitative measurement shows higher phenol and flavonoids content in leaf compare to stem during summer season. So, summer season is the best and leaf is the best plant part for the collection of some phytochemicals in *Tecomella* plant.

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