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Qualitative analysis, total phenol content (TPC) and total tannin content (TTC) by using different solvant for flower of *Butea monosperma* (Lam.) Taub. collected from Saurashtra region

Milan Vala and Bharat Maitreya

Abstract
Butea monosperma (Lam.) Taub. is a member of *Leguminosae* (Fabaceae) family and sub family *Papilionaceae*. Present study showed that qualitative analysis, quantitative estimation for Total Phenol Content (TPC) and Total Tannin Content (TTC) and FTIR analysis for flower of *Butea monosperma* from Saurashtra region of Gujarat state. The phytochemical analysis was performed using two different solvents included Acetone and ethanol. It's revealed that various bioactive constituents were present which are important in medicinal level. Flowers were useful for diuretic, tonic depurative, astringent, leprosy, gout, burning sensation.

Keywords: *Leguminosae*, phenol, tannin, quantitative estimation, *fabaceae*

Introduction
Nature is a unique source of structures of high phytochemical diversity (Saxena *et al*., (2013)) [10]. Medicinal plants are very important for health and its play definite physiological action on human body (Ahmad T, Singh *et al*., (2013) [9]. *Butea monosperma* (Lam.) belongs to *fabaceae (leguminosae)* family and subfamily *papilionaceae.* It is also known as Flame of the forest. Commonly it's called as khakharo or kesudo. *Butea monosperma* is a medium sized deciduous tree, trunk crooked and irregular branches and rough. Its height is 12-15 m with gray flaky bark. Leaves are alternate, large, pinnately trifoliate, spreading, long stalked and petiolate. Flowers are large and bright orange red in colour. Orange colored blooms appear during February-April. Flower buds appear in January. Pods ripen in May-June. Pod size is 4-6 inches long, oblong, blunt, as a fruit pod with a single seed in each (Geeta R *et al*., (2011) [2]. *Butea monosperma* is most important and used as a tonic, astringent, diuretics, aphrodisiac, inflammations, bleeding piles, eye disease, skin disease, tumors, abdominal discomfort etc. (Thooyavan G & Karthikeyan J (2016) [7]. Flowers are depurative, as a poultice. They are important for disperse swelling (Shrirao AV *et al*., (2017) [8]. Quantification of metabolites will help for extraction; purification and identification of several different bioactive compounds for use various aspects (Santhi K and Sengottuvel R (2016) [12]. Flowers are effective against liver disorders and also reported to possess anti-implantation activity (Kumar DM *et al*., (2017) [3]. The full form of FTIR is Fourier Transforms Infrared Spectroscopy. OPUS software was used to acquire and manipulate the spectral data. FTIR is very fast method of analysis (Goldson A. *et al*. (2016) [11].

Material and Methods
Collection of plant materials
Fresh flower of *Butea monosperma* were collected from Amreli district of Saurashtra. The flowers of plant were collected and washed with tap water and again it with distilled water. Flowers were dried at room temperature and then crushed. Dried powder stored in the air tight bottle for further analysis.

Preparation of plant extract
10 gm of plant powder were added into 100 ml selected solvents (Acetone and Ethanol). Then shaken well and kept it overnight for soaked. After 24 hours filter the samples through whatman filter paper no.1 and these filtrate was collected in petriplates and allowed it till solvent was evaporated. Collected the extract and stored it 20 °C for further analysis.

Phytochemical analysis

The qualitative phytochemical analysis of acetone and ethanol crude extracts by cold extraction method was individually performed for the presence of various bioactive constituents' phenols, tannins, carbohydrates, alkaloids, proteins, amino acids and glycosides etc by standard procedure. Quantitative estimation of Total Phenol Content (TPC) and Total Tannin Content (TTC) were also done.

Qualitative analysis [Panchal PM (2012) [5], Banu KS and Cathrine L (2015) [1]

Phenols and tannins:

1) **Test of 5% $FeCl_3$:** 50 mg of extract is dissolved in 5ml of distilled water. To this a few drops of freshly prepared 5% $Fecl_3$ solution was added. Blue-green color indicated presence of phenolic compounds.
2) **Lead acetate test:** The 50 mg extract was dissolved in of distilled water and to this 3 ml of 10% lead acetate solution was added. A bulky white precipitate obtained.
3) **Test with $KMnO_4$:** Few ml extract was taken and 1 ml potassium dichromate solution was added. Precipitation indicated presence of tannins and phenolic compounds.

Quantitative analysis

Total Phenol Content (Vaidya A and Nancy P (2017)) [6]

Quantitative analysis of total phenol contents (TPC) of *Butea monosperma* flower were done by folin ciocalteau's method with some modifications. For the prepare calibration curve, gallic acid was used as a standard. Gallic acids were prepared in different solvent (ethanol and acetone) with different concentration. A volume of 0.5 ml of each concentration of gallic acid was mixed with 0.2 ml of (1:10) Folin ciocalteau's reagent and 2 ml of 7.5% sodium carbonate solution then the tubes were shaken vigorously and mixed well. The reaction mixture was incubated for 30 minutes at room temperature and absorbance was measured 760nm using spectrophotometer. Same as for plant extract, 0.5 ml of all extracts (1 mg/ml) were treated and absorbance was measured.

Total Tannin Content (Padma R *et al.,* (2013)) [4]

Quantitative analysis of total tannin content (TTC) of *Butea monosperma* by folin denis method with some modifications. Tannic acid was used as standard. Tannic acid was prepared with different concentration with different solvents. The ethanolic and acetone extract mixed with 0.1 ml folin denis reagent (1:10) then 1 ml sodium carbonate (7.5%) was added. These mixtures shaken well and allowed it to 30 minutes for incubation at room temperature and measured the absorbance at 700 nm using UV-visible spectrophotometer. Total tannin content was calculated as mg tannic acid equivalent from equation obtained from a calibration curve.

Results and Discussion

The powedered of *Butea monosperma* flower extracted using different solvents (Acetone and Ethanol).

Table 1: Qualitative Phytochemical screening of *Butea monosperma* flower extracts

No.	Name of metabolites	Test name	Saurashtra	
			***Butea monosperma* Flower (Ethanol)**	***Butea monosperma* Flower (Acetone)**
1.	Alkaloids	a) Mayer's test	-	++
		b) Dragendroff test	-	++
		c) Wagner's test	-	++
2.	Flavonoids	a) Zinc hydrochloride reduction test	+	+
		b) Pew test	++	+
3.	Phenols	a) Ferric chloride test	++	+
		b) Lead acetate test	+	+++
4.	Tannins	a) Potassium dichromate test	+	++
		b) Lead acetate test	+	+++
5.	Steroids	Libermann-sterol test	+	+
6.	Glycosides	Keller-killani test	+	+
7.	Sugar/Carbohydrates	a) Molisch's test	++	-
		b) Fehling's test	-	-
		c) Benedict's test	-	-
8.	Protein/ Amino acid	a) Millon's test	++	++
		b) Ninhydrin test	-	-
		c) Xanthoproteic test	-	-
9.	Fixed oil		-	-

Here – (Not present), + (Slightly present), ++ (Quite present), +++ (Highly present)

Qualitative phytochemical analysis

Phytochemical analysis of *Butea monsperma* flower has been done for the selected Saurashtra regions of Gujarat. Different solvents were used for extraction. Qualitative phytochemical analysis of *Butea monsperma* flower revealed that presence of various bioactive constituents. In Acetone solvent contained alkaloids, phenols, tannins, steroids, glycosides, protein/amino acid etc were present while flavonoids, phenols, tannins, steroids, glycosides, sugar/carbohydrates, protein/amino acid etc were present in ethanolic solvent. Different environmental factors also effect on plant constituents such as rain fall, climate change and some other edaphic factors.

Table 2: Total Phenol Content for *Butea monosperma* Flower

Total Phenol Content (TPC)						
Butea monosperma	**Extract**	**Concentration (mg/ml)**				
		0.1	**0.2**	**0.3**	**0.4**	**0.5**
Saurashtra	Acetone	0.253±0.016	0.488±0.008	1.079±0.012	1.113±0.012	1.152±0.016
	Ethanol	0.059±0.001	0.180±0.005	0.239±0.004	0.330±0.003	0.446±0.001

Table 3: Total Tannin Content for *Butea monosperma* flower

Total Tannin Content (TTC)						
Butea monosperma	**Extract**	**Concentration (mg/ml)**				
		0.1	**0.2**	**0.3**	**0.4**	**0.5**
Saurashtra	**Acetone**	0.258±0.002	0.368±0.002	0.385±0.002	0.394±0.002	0.438±0.005
	Ethanol	0.315±0.004	0.462±0.015	0.517±0.025	0.924±0.002	1.111±0.009

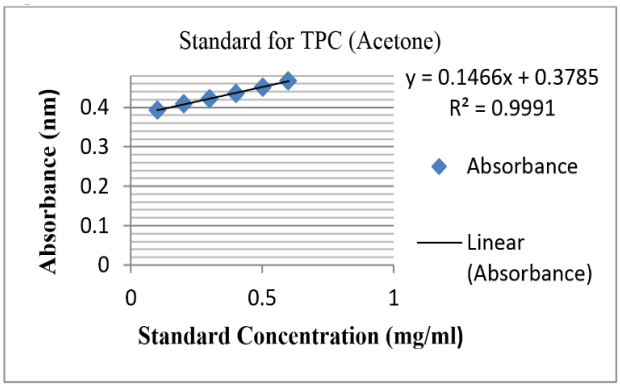

Fig 1: Gallic acid standard graph (Acetone)

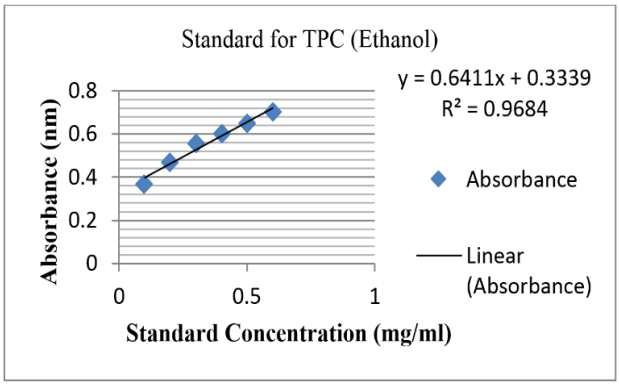

Fig 2: Gallic acid standard graph (Ethanol)

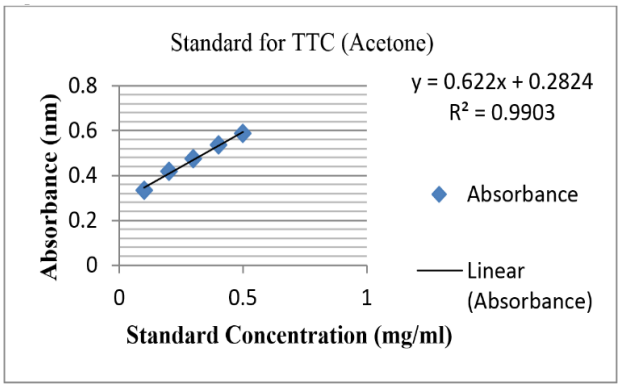

Fig 3: Tannic acid standard graph (Acetone)

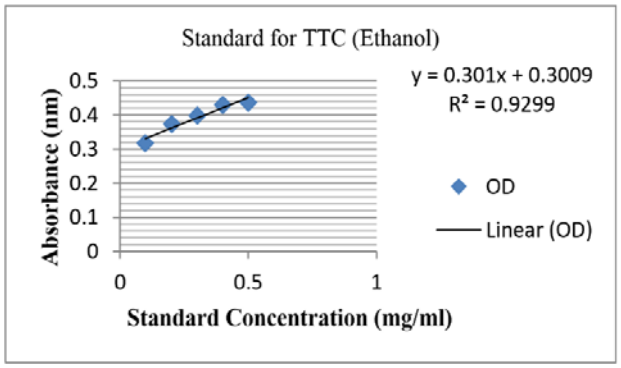

Fig 4: Tannic acid standard graph (Ethanol)

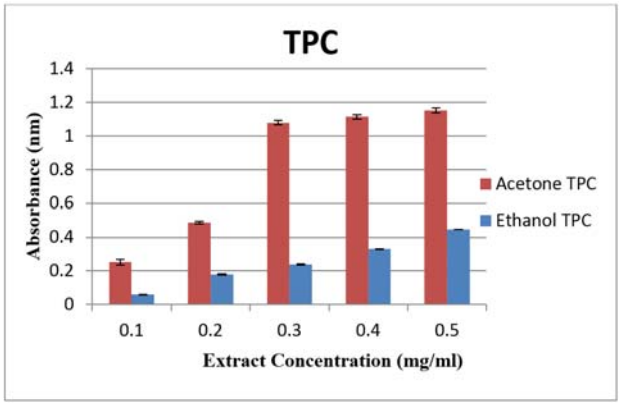

Fig 5: For *Butea monosperma* flowers Total Phenol Content (Saurashtra)

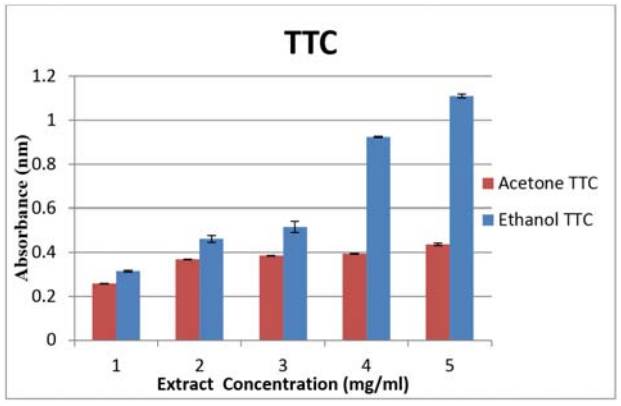

Fig 6: For *Butea monosperma* flowers Total Tannin Content (Saurashtra)

Quantitative phytochemical analysis

Total phenol content of *Butea monopserma* flower was estimated by folin-ciocalteau method. TPC was calculated for acetone extract from regression equation of calibration curve (y= 0.1466x+0.3785, R^2 =0.9991) and for ethanol solvent from regression equation of calibration curve (y=0.6411x+0.3339, R^2=0.9684) and total phenol content (TPC) was expressed as Gallic acid equivalents (GAE). In Saurashtra region *Butea monosperma* flower, Acetone extract showed total phenol content (TPC) 1.152±0.016 and ethanol extract showed total phenol content 0.446±0.001 mg/ml of gallic acid equivalent (GAE).

In Total tannin content of *Butea monosperma* flower was calculated for acetone solvent from regression equation of calibration curve (y= 0.622x+0.2824, R^2 =0.9903) and for ethanolic solvent from regression equation of calibration curve (y=0.301x+0.3009, R^2=0.9299) and total tannic content (TPC) was expressed as mg of tannic acid equivalents per gm of extract. In Saurashtra region *Butea monopserma* flower acetone extract showed total tannin content (TTC) 0.438±0.005 and ethanol extract showed total tannin content 1.111±0.009 mg/ml of tannic acid equivalent.

FTIR analysis

Based on FTIR analysis, in FTIR spectra of *Butea monosperma* acetone flower extract the strong peaks observed at 3468.25 cm-1(O-H stretch in alcohol), 3005.62 cm-1(O-H stretch in carboxylic acid), 2148.25 cm-1(N=C=S stretch of isothiocyanate), 1994.38cm-1 (N=C=S stretch of isothiocyanate), 1704.45 cm-1(C=O stretch of conjugated acid dimer), 1421.56 cm-1 (O-H bending in carboxylic acid), 1360.32 cm-1(C-F stretching in fluoro compound), 1223.72 cm-1(C-F stretching in fluoro compound), 1092.70 cm-1(C-O stretch of secondary alcohol), (905.38 cm-1, 612.71 cm-1, 531.01 cm-1(Trisubstitution)) while in ethanolic extract of flower 3328.69 cm-1(O-H stretching of alcohol), 2973.30 cm-1(O-h stretch in carboxylic acid), 2883.85 cm-1 (C-H stretch of alkane), 2123.75 cm-1(N=C=S stretch of isothiocyanate), 1992.38 cm-1(N=C=S stretch of isothiocyanate), 1924.41 cm-1(N=C=S stretch of isothiocyanate), 1657.99 cm-1(C=C stretch in alkane), 1451.72 cm-1(C-H bending of alkane in methyl group), 1417.70 cm-1(O-H bending in Carboxylic acid), 1379.84 cm-1(O-H bending of phenol), 1329.70 cm-1(O-H bending of phenol), 1274.27 cm-1(C-F stretch in fluoro compound), 1086.85 cm-1(C-O stretch of aliphatic ether), 1044.75 cm-1(CO-O-CO stretch in anhydride), 879.61 cm-1, 803.32 cm-1, 635.02 cm-1, 578.84 cm-1(Trisubstituted).

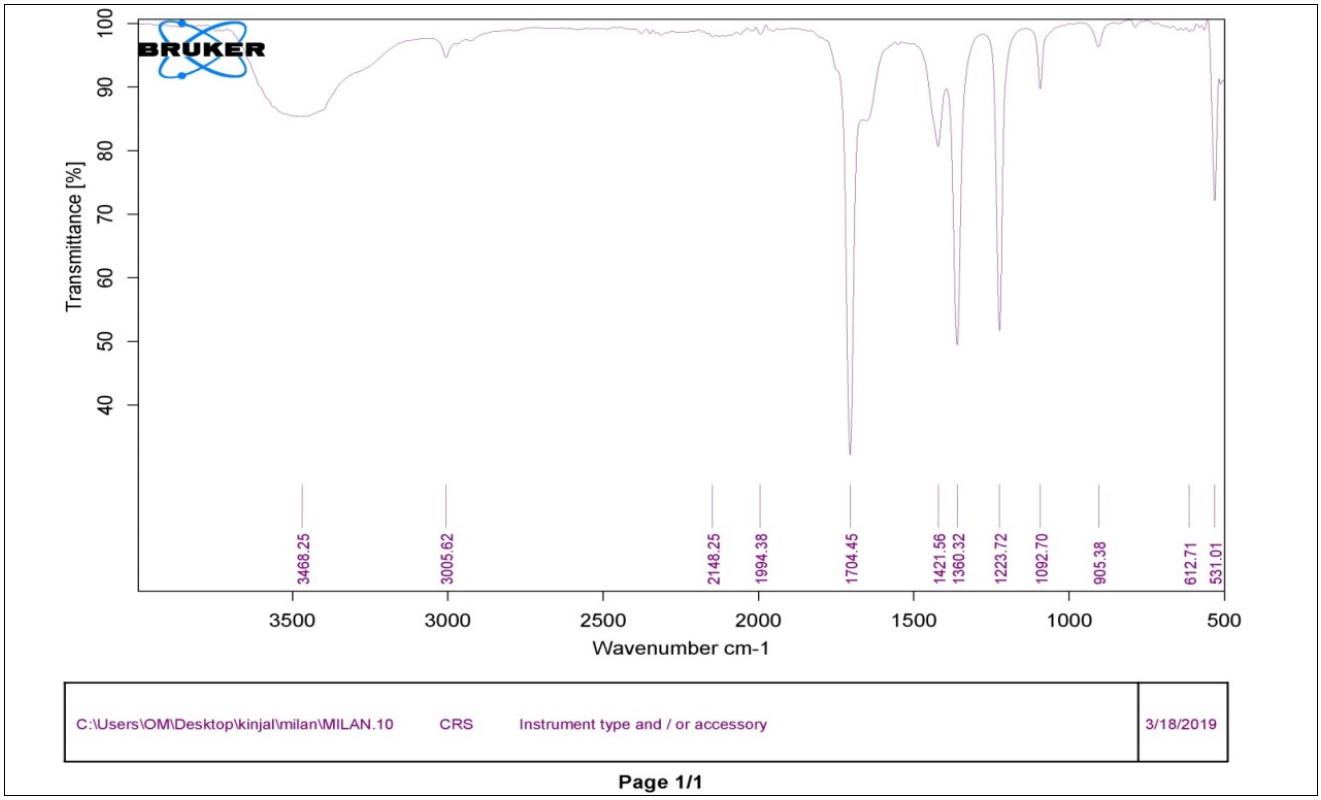

Fig 7: FTIR analysis for *Butea monosperma* flower (Saurashtra) Acetone

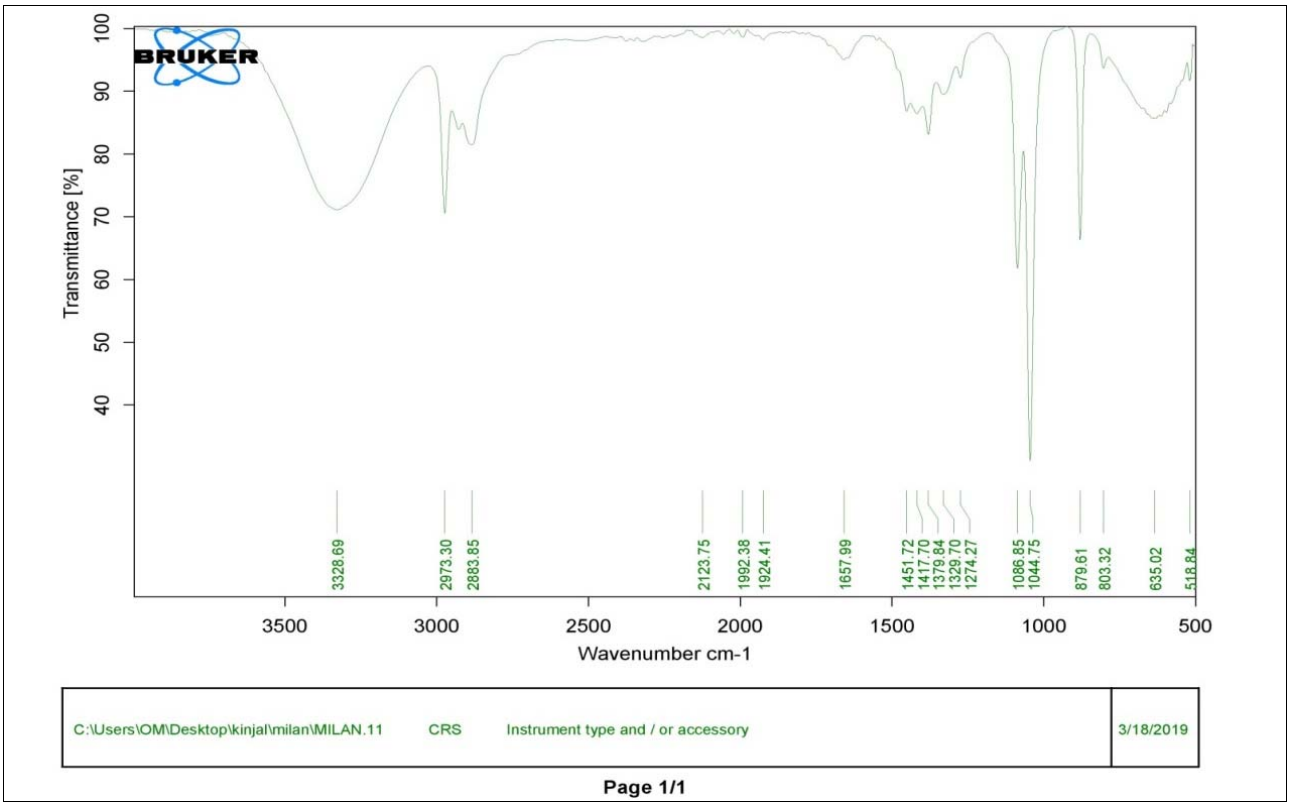

Fig 8: FTIR analysis for *Butea monosperma* flower (Saurashtra) Ethanol

Conclusion

Present study showed that, Based on phytochemical analysis various bioactive constituents such as alkaloids, phenols, flavonoids, tannins, steroids, glycosides and sugar/carbohydrates were present which have good medicinal value. Generally medicinal plant play vital role in prevent various diseases. Thus it is important for medicinally as well as in new drug development. *Butea monosperma* are important for dying color for fabric, as pesticides, as ailments, anti-fungal activity, and antifertility activity.

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