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Comparative preliminary phytochemical investigation and total flavonoid content estimation in *Cymbopogon citratus* Stapf, *Ocimum sanctum* Linn. and *Trigonella foenum-graecum* Linn.

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Abstract
The objective of the present study is to evaluate and compare the phytochemical constitution of methanolic and petroleum ether extracts and determination of total flavonoid content of three medicinally important herbs *Cymbopogon citratus*, *Ocimum sanctum* and *Trigonella foenum-graecum*. The phytochemical analysis was performed by standard methods to detect the presence of glycosides, saponins, tannins, phenolics, alkaloids, flavonoids, triterpenoids and steroids. The total flavonoid content (TFC) was accessed at 510 nm using Rutin as standard and the calculated amount is found higher in Ocimum sanctum.

Keywords: *Ocimum sanctum, Cymbopogon citratus, Trigonella foenum-graecum,* flavonoid

Introduction
Since ancient times herbal medicines has been used in treatment of variety of diseases. It is their primary and secondary metabolites which provides medicinal properties. *Cymbopogon citratus*, *Ocimum Sanctum Linn.* and *Trigonella foenum-graecum* are very commonly used in our daily life and have wide range of applications. Keeping in view the importance of these plants the present study is designed to compare the phytochemicals present in them and to estimate the total flavonoid content (TFC) in the three medicinal plants belonging to different families. TFC is measured spectrophotometrically using Rutin as standard and expressed as Rutin equivalent (RE).

Materials and methods
Collection of plant materials
Fresh leaves of medicinal plants, *Cymbopogon citratus*, *Ocimum sanctum and Trigonella foenum–graecum* were collected from local areas of Bhopal. The plant material was taxonomically identified by the Head, Department of Botany, Saifia College of Science, Bhopal. The plant material was shadow dried and then grinded coarsely using mechanical grinder.

Preparation of plants extracts
Crude extracts of leaves of plant was prepared by cold maceration method. It was firstly defatted by petroleum ether and then extraction with alcohol was done. Dried extract was then kept in refrigerator at 4^{o}C for further use in analysis.

Qualitative phytochemical analysis
Phytochemical analysis was performed to identify presence or absence of different phytoconstituents by standard methods (C.K. Kokate, *et al*., 2006)

Test for glycosides
Borntrager's test
Make an acidic solution of test using dilute sulphuric acid, it is boiled and filtered. To the cold filterate, equal volume of chloroform was added and shaken. The organic layer was separated and ammonia was added to it. Formation of pink to red colour in ammoniacal layer indicates presence of anthraquinone glycosides.

Legal's test
1 ml of test solution was dissolved in pyridine.1ml of this sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Test for saponins
Froth test
The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The appearance of layer of foam indicates the presence of saponins.

Test for tannins and phenolic compounds
Ferric chloride test
Make aqueous solution of extract. To this add 2 ml of 5% ferric chloride solution. Formation of blue, green or violet colour indicates the presence of phenolic compounds.

Lead acetate test
In aqueous solution of extract add few drops of lead acetate solution. Formation of white precipitate indicates presence of phenolic compounds.

Test for alkaloids
Extract was made acidic by adding dilute hydrochloric acid, it

was mixed and filtered with the filtrate following test were performed.

Mayer's test
In 4 ml of filtrate, few drops of Mayers reagent were added along sides of tube. Whitish or creamy precipitate indicates the presence of alkaloids.

Hager's test
To 4 ml of filtrate few drops of Hager's reagent were added in a test tube. Red precipitate indicates the presence of alkaloids.

Test for flavonoids
Lead acetate test
The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate is indicative of the presence of flavonoids.

Alkaline reagent test
The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which gradually becomes colourless on addition of few drops of dilute acid, indicate presence of flavonoids.

Test for Triterpenoids and steroids
Salkowski's test
The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterols are present. But if it is golden yellow layer at bottom indicates the presence of triterpenes.

Libermann-Burchard's test
The extract was treated with chloroform. To this solution few drops of acetic anhydride were added it was then boiled and cooled. To this cold solution concentrated sulphuric acid was added through the sides of the test tube carefully. Formation of brown ring at the junction of two layer, green upper layer indicates presence of steroids and formation of deep red colour is indication of presence of triterpenoids.

Total Flavonoid Content Estimation
(Zhishen J. *et al* 1999) [14]: In the preliminary studies the methanol extracts of plants shows the presence of flavonoid, they were analysed quantitatively. For this different concentration of Rutin was prepared in methanol. The test sample (methanolic extract) is prepared in methanol (100 µg/ml). By mixing 0.5-ml aliquot of appropriately diluted sample solution with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution, it was kept for 6 minutes. After 6 min, add 0.15 ml of a 10% $AlCl_3$ solution and allow to stand for 6 min, then add 2 ml of 4% NaOH solution to the mixture. Now, add water bringing final volume to 5 ml, and then mix the mixture thoroughly and allowed to stand for another 15 min. Finally absorbance of the mixture at 510 nm was taken versus prepared water blank. The analysis was done in triplicate for each extract for more accurate results.

Results

Table 1: Preliminary Phytochemical Screening.

	Plants					
	Cymbopogon citratus		***Ocimum sanctum***		***Trigonella foenum-graecum***	
	PE	**ME**	**PE**	**ME**	**PE**	**ME**
Glycosides	--	--	--	+	--	--
Saponins	--	+	--	+	--	+
Tannins and Phenolics	--	--	--	+	--	--
Alkaloids	--	--	--	--	--	--
Flavonoid	--	+	--	+	--	+
Triterpenoids	+	--	+	--	+	--
Steroids	+	+	+	+	+	--

+ = Presence, -- = Absence, PE= Petroleum ether extract, ME = methanolic extract

Table 2: Standard reading of different concentration of Rutin

S. No	**Conc. (µg/ml)**	**Absorbance**			**Mean Value**
1	10	0.136	0.133	0.137	0.135
2	20	0.150	0.147	0.152	0.149
3	30	0.162	0.163	0.165	0.163
4	40	0.175	0.177	0.176	0.176
5	50	0.197	0.205	0.204	0.202

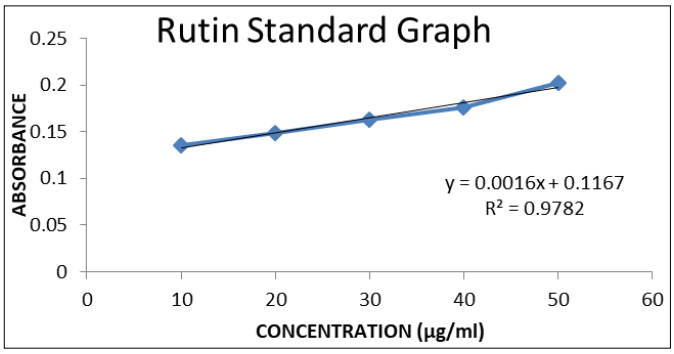

Fig 1: Standard graph of Rutin

From the standard curve of Rutin the line of regression was found to be:-

$$y = 0.001x - 0.116 \text{ and } R^2 = 0.978$$

Line of regression was used for estimation of unknown flavonoid content by putting the absorbance "Y" of test sample in line of regression of standard curve of Rutin.

Table 3: Flavonoid Content estimation

S. No.	Plant	Conc.	TFC (mg RE/g)	SD
1	*Cymbopogon citratus*	1 mg/ml	31.33	±.577
2	*Trigonella foenum graecum*	1 mg/ml	117.66	±.577
3	*Ocimum Sanctum*	1 mg/ml	265	± 1

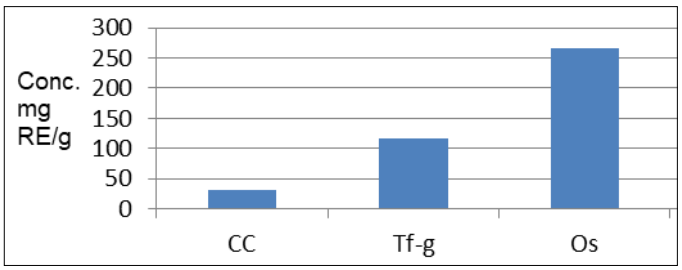

Fig 2: Graphical Representation of Conc. Of Flavonoid content (mg RE/g)

It can be seen that the TFC is much higher in *Ocimum sanctum* among others. There is a significant difference which is clearly evident from their Figure representation.

Discussion

The phytochemical constituents is summarized in Table 1. In previous study significant antimicrobial extract of tulsi has been observed (Singh *et al*., 2015) [15] (Joshi *et al*., 2011) [5] The other species of tulsi, *Ocimum gratissimum linn., Ocimum americanum linn., and Ocimum basilicum* has also been reported to contain the bioactive compounds and suggests its importance in medicinal use. The level of constituents also vary with time and cultivation process (Tiwari *et al*. 2012) [13]. The GC-MS analysis has revealed that *Ocimum Sanctum* leaves contains mainly eugenol and caryophyllene (Devendran *et al*., 2011) [4] When compared to aqueous extract methanol extract of tulsi possess more phytoconstituents (Sadul *et al*., 2015) [10]. Studies has also shown that leaves and stems of tulsi has almost same phytochemicals (Shafqatullah *et al*., 2013) [10] A wide range of chemical compounds including eugenol, euginal, urosolic acid, carvacrol, caryophyllene are also present in tulsi (Rahman *et al*., 2011) [8] Studies has also showed the presence of flavonoids, phenolic compounds, glycosides amd conjugated dienes in lemon grass (Akande *et al*., 2012) [1]. Its essential oil are considered safe for human consumption (Christopher *et al*., 2014) [3] GC-MS analysis of its essential oil has demonstrated that it is dominated by monoterpene hydrocarbons (Kimutai A. *et al*., 2017) [6] It is also used as antidiabetic, anti-fertility, anti-microbial, antiparasitic, antiepileptic, antibronchitis, carminative, aphrodisiac (Patil S 2014) [7]. The flavonoids show a strong antioxidant properties, so as a comparative analysis *Ocimum Sanctum* is a strong antioxidant content plant among the three.

Conclusion

The present study revealed that methanolic extract of *Cymbopogon citratus* contain carbohydrates, saponins, flavonoids and steroids. The extract of O*cimum sanctum* contain carbohydrates, glycosides, saponin, tannin, flavonoid and steroid. The extract of *Trigonella fornum-graecum* contains carbohydrates, saponins, flavonoids, and tannins. The petroleum benzine extract of all the three plants contains triterpenoids and steroids. Thus illustrating the richness of *Ocimum sanctum* extract over *Cymbopogon citratus and Trigonella foenum-graecum*. The TFC results also shows that the highest content is in *Ocimum sanctum* as compared to *Trigonella fornum-graecum* and *Cymbopogon citratus.*

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