CHARACTERIZATION OF FLAVONOIDS ISOLATED FROM *Trigonellafoenum-graecum* USING UV SHIFT REAGENT

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ABSTRACT

The biggest challenge in utilizing plant secondary metabolites lies in extraction, isolation and characterization using available analytical techniques. UV absorption is an excellent technique for the identification and characterization of phenolic compounds. Phyto compounds characterization using UV absorption spectrum is very helpful and it gives quick results which can be interpreted easily, wherein preparation of the sample for analysis involves no difficulty. In this study, we have identified and characterized 2 flavonoids, kaempferol 3-o rutinoside and chrysoreol using UV shift reagents, isolated from *Trigonella foenumgraecum* methanolic seed extract. The same was confirmed by reference Mass and NMR spectral results reported in the literature. Characterization using UV-visible spectrometry is a powerful technique that can be employed for phytochemical identification and characterization purposes with great leverage when sample isolated is less.

Keywords: UV Characterization of Flavonoids, *Trigonella foenumgraecum*, Shifts Reagent, Flavones

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INTRODUCTION

Phytocompounds are available in various plant materials and show potential biological activities in the treatment and prevention of cancer, cardiovascular, diabetes and other chronic diseases.¹⁻³ Alkaloids, tannins, flavonoids, and phenolic compounds are the most important among the phytocompounds.⁴ These are major phytocompounds that are proved in various studies for the treatment of cardiovascular chronic diseases.^{3,5-9} Flavonoids are phenolic compounds that originate from phenyl propanoid pathway¹⁰, serve as antioxidants, present in wide varieties of plants from roots, fruits, seeds etc¹¹ and more than 4000 flavonoids are identified till now.¹² Isolated compounds from *Trigonella* like Quercetin, Naringenin, Luteolin and Chrysin have anti-depressant activity¹³ and seed extract of *Trigonella* has significant anti-corrosive activity.¹⁴ They are the most analyzed group of phytocompounds because of their unique coloring characteristics and anti-oxidant properties.¹⁵ The biggest challenge in isolation of flavonoids lies in the method of extraction based on solvents polarity involving sequential solvent extraction with number of solvents, suitable isolation technique, and then its identification and characterization.^{16,17}

No single analytical technique is enough for the identification and characterization of isolated compounds.¹⁷ It needs high instrumentation techniques like MS/MS, NMR where sample preparation is so critical. A combination of different qualitative and quantitative techniques is required for confirmation of the structure of the compound.¹⁸

They consist of 15 carbon compounds that have a carbon skeleton in C₆-C₃-C₆ manner.¹⁰ These secondary metabolites are widely distributed among all lower and higher vascular plant species, bryophytes, and algae.¹⁹⁻²¹ They can be identified by analytical tests like Shinoda, sodium hydroxide and p-dimethyl amino cinnamaldehyde tests.¹⁷

In this paper, flavonoids isolated from methanolic extract of seeds of *Trigonella foenumgraecum* were characterized using Shift reagents of UV.

EXPERIMENTAL

Material and Methods

The selected plant material was obtained from Natural Remedies Pvt. Ltd. Bangalore. It was identified as *Trigonella foenumgraecum* by a qualified botanist.

Extraction of Plant Material using Methanol

40 kg of powdered dried seeds of *Trigonella foenumgraecum* were taken for extraction using methanol as solvent.²² Detailed information on the extract is given in Table-1.

Table-1: Preliminary information of Seed Extract

S. No.	Samples	Sample taken	Colour	Taste	Consistency	%Yield
1	Methanolic extract	37.03 kg	Dark brown	Bitter	Dried lumps	13.5%

Instrument

UV visible spectrophotometer (Make: Shimadzu UV1650PC)

Isolated compounds were dissolved in methanol solution and mixed well, to measure the spectrum using shift reagents.²⁴

Preparation Method of U.V Shift Reagents

Sodium Methoxide Solution

About 2.5g of metallic sodium weighed and transferred to 100mL methanol.

Sodium Acetate

Anhydrous sodium acetate is used.

Boric Acid

Anhydrous Boric acid powder is used.

Aluminium Chloride

About 5g of aluminium chloride weighed and transferred to 100mL of methanol.

HCl

50mL concentrated HCl added to 100mL water.

Preparation of Shift Reagent 1

(Methanolic Sample + Sodium methoxide)

First, a flavonoid in methanol solution was transferred to cuvette and spectrum was measured. About 3 drops of sodium methoxide were added to the cuvette, mixed well and spectrum were measured. Cuvette was washed thoroughly before proceeding to the next step.

Preparation of Shift Reagent 2

(Sample Solution + Aluminium chloride; HCl Solution)

About 6 drops of aluminium chloride were added to the sample solution in cuvette and spectrum was measured. To this, HCl (3 drops) was added and the spectrum was recorded.

Preparation of Shift Reagent 3

(Sample Solution + Sodium acetate; Boric Acid)

The fresh sample solution was taken into cuvette and anhydrous sodium acetate powder was added to the cuvette to form a layer (1mm) at the bottom. Spectrum was measured. After adding Boric acid (quantity same as sodium acetate powder) to this solution, the spectrum was recorded again.

RESULTS AND DISCUSSION

UV Spectrum with Shift Reagents

In UV spectroscopy, flavonoids show two absorption maxima, band-I (at 300 nm-350 nm) and band II (at 240 nm-285 nm) from A and B rings respectively. The presence of hydroxyl groups at different positions in the flavonoid A and B rings are the criteria for the classification of flavonoids. It can be identified by using UV shift reagents Sodium methoxide (NaOMe), Aluminium chloride (AlCl_3), Hydrochloric acid (HCl), Sodium acetate (CH_3COONa) and Boric acid (H_3BO_3)

Fig.-1: The Spectrum of Sample 1 in Methanol

Fig.-2: Spectrum of the Sample 1 with Shift Reagent 1

Fig.-3: The Spectrum of Sample 1 with Shift Reagent 2

Fig.-4: Spectrum of the Sample 1 with Shift Reagent 2 + HCl

Fig.-5: Spectrum of Sample 1 in Methanol with Shift Reagent 3

Fig.-6: Spectrum of Sample 1 in Methanol with Shift Reagent 3 + H_3BO_3

Fig.-7: Spectrum of Sample 2 in Methanol

Fig.-8: Spectrum of Sample 2 with Shift Reagent 1

Fig.-9: Spectrum of Sample 2 with Shift Reagent 2

Fig.-10: Spectrum of Sample 2 with Shift Reagent 2 + HCl

Fig.-11: Spectrum of Sample 2 with Shift Reagent 3

Fig.-12: Spectrum of Sample 2 with Shift Reagent 3 + H₃BO₃

Interpretation From Each Step of Shift Reagent Addition

Step-1: Gives Spectrum which indicates 3 hydroxy, 4 hydroxy and 7 hydroxy groups in the Flavonoid Compound (Shifts in Band II)

Sodium methoxide causes hydroxylation of phenyl groups which leads to bathochromic shift in the band I by 40-80nm. It represents free -OH groups present in 4' position. Less alkali sensitive groups get substituted while more acidic groups remain in unsubstituted form. In this step, 4 hydroxy and 7 hydroxy groups substitution can be detected. The presence of 4 hydroxy groups in the sample compound causes

bathochromic shift (shift towards longer wavelength) with an increase in intensity. The presence of alkali-sensitive groups causes degradation of the spectrum after some time. Both bathochromic shift and decrease in intensity indicate no free 3, 4, 7 hydroxy groups. A shift of 50 nm indicates the presence of 3 hydroxy groups.^{24,25}

Step-2: Gives Spectrum which indicates the presence of ortho di hydroxy groups in Flavonoid Compound (Shifts in Band I and Band II)

After adding acid to the aluminium chloride sample solution ss in both band I and band II represents chelated -OH at 5 positions. It also shows a hypsochromic shift, (decrease in wavelength) that indicates the presence of ortho dihydroxy groups in the sample.²⁴

Step-3: Gives Spectrum which indicates Free 7 Hydroxy Groups (Shift in Band II)

When sodium acetate is added to the methanolic solution of the flavonoid sample, a 12 nm bathochromic shift in band II indicates the presence of ortho dihydroxy groups in the flavonoid compound.²⁴

1. In Fig.-1 and 2, we can see a bathochromic shift of 60 nm in the band I, with little change in intensity after the addition of sodium methoxide. It indicates a 'flavone' with a free -OH group in position 4'.²⁶
2. Highly oxygenated compounds absorb at longer wavelengths than those of the fewer oxygenated substituents.
3. Methylation and glycosylation of free hydroxyl groups cause hypsochromic shift in the band I
4. Acetylation of flavonoid nullifies the effect of phenolic hydroxyl groups on the spectra. It is a valuable technique for locating alkoxyl groups.
5. The addition of base to the free phenolic hydroxyl groups in flavonoid causes ionization, makes a shift of wavelength towards longer wavelengths. This extension of bathochromic shift depends on the presence of overall substitutions.

These results from UV spectrum recorded by using shift reagent shows that isolated compounds belong to the 'flavone' group of flavonoids, which were confirmed by Electron Ionization Mass Spectra (EIMS), IR and NMR spectra results. The isolated flavones identified as 'Kaempferol 3-O-rutinoside' and 'Chrysoreol'.^{22,27}

CONCLUSION

Using UV-shifts reagents for the identification of flavonoids is an easy technique but needs confirmation by other techniques as well. Before going for higher instrumentation techniques for characterization, we can rely on a UV spectrophotometer to characterize the flavonoids. In this study, as isolated flavonoid sample quantity was very less. Confirmation of identified flavonoids was done by mass, IR and NMR spectra, but results were not good enough to do for reliable characterization. Even with a small sample quantity, UV identification method by shift reagent gave better results. Shift reagents for flavonoid characterization are quick and reliable as there is no difficulty in sample preparation.

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