Eurasian Journal of Analytical Chemistry
ISSN: 1306-3057OPEN ACCESS 2017 12(7b):1673-1679

Functional Group Analysis of Various Extracts of Desmodium Gangeticum by FT-IR Spectrum

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Received 1 January 2017 ▪ Revised 10 February 2017 ▪ Accepted 24 February 2017

Abstract: *A large number of medicinal plant and their purified constituents have shown beneficial thereupatic potentials. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds,it is important to thoroughly investigate theiruse. Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs.The present study dealt with the qualitative preliminary phytochemical screening of Desmodiumgangeticum was done. Qualitative preliminary phytochemical analysis was performed in aqueous, chloroform and ethanolic extract of Desmodiumgangeticum. The chloroform extract contains more phytochemical constituents when compared to other extracts. The functional group in Desmodiumgangeticum also confirmed by FT-IR spectroscopic technique. Some phytoconstituent were separated in Desmodiumgangeticum extract by thin layer chromatography technique.*

Keywords: *Desmodiumgangeticum, Column chromatography, TLC and FT-IR.*

INTRODUCTION

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate alternative sources for the treatment of resistant strains. Presently 80 percent of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases.

Many plants synthesize substances that are useful for the maintenance of health in human and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins.In many cases, substances such as alkaloids serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many of the herbs and spices used by human to season food yield useful medicinal compounds. **[Nirmaladevi and Padma 2008]**

Natural compounds extracted from plants, particularly higher plants, have been suggested as an alternative source for antibiotics. The chemical features of these constituents differ considerably among different species. This approach is alluring, in part, because they constitute a potential source of bioactive compounds that have been professed by the general public as comparatively safe and often act at multiple and novel target sites, thereby reducing the potential for resistance.

MATERIALS AND METHODS

Collection and Authentication ofPlant Material

The leaves of *Desmodiumgangeticum* were collected in the month of August from the local field of Lalgudi, Trichy, India. Before the initiation of extraction the collected leaves were authenticated and deposited in the RAPINAT HERBARIUM, St.Joseph College, Tiruchirappalli.

Leaves were shade dried, coarsely powdered with an electrical blender, dried leaves were ground into coarse powder and were first defatted with ethanol which is further evaporated to dryness and utilized throughout the study.

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Preparation of Different Fractions of *DesmodiumGangeticum*

Chloroform, alcoholic fractions and aqueous extract were obtained by standard protocolsas describedbyHarborne*et al.,* 1999.

Chloroform Extraction

Coarse powder of the medicinal plant *Desmodiumgangeticum* leaves was taken and weighed separately up to 100 grams and mixed with 300 ml of Chloroform. Beaker was closed with aluminum foil and left for 72 hours at room temperature. The extract was filtered through three layered muslin cloth and condensed into the powder by evaporation in water. The condensed powder was stored at 4o C and utilized throughout the studies (Harborne et al.,1999).

Alcohol Extraction

The coarse powder of *Desmodiumgangeticum* which was already extracted with chloroformwas added with 300 ml of alcohol. Beaker was closed with aluminum foil and left for 72 hours. The extract was filtered, and processed. Fraction obtained was weighed to find out the extraction values.

Aqueous Extraction

Coarse powder of the medicinal plant *Desmodiumgangeticum* leaves was taken and weighed up to 100g and dissolved in 300ml of sterile distilled water. This substance was boiled for 30 minutes. Then the extract was filtered through three layered muslin cloth and condensed in to solid form at 40o C using hot air oven. The extract was weighed to find out the extraction value, and stored in a sterile container at 4^{o} C for further use.

TLC profile

Thin layer chromatography is an important tool in the separation, identification and estimation of different components. Here the principles of separation are adsorption and the stationary phase acts as an adsorbent. Depending on the particular type of stationary phases, its preparation and use with different solvents can be achieved on the basis of partition and adsorption. The plant extracts showed good resolution in solvent system by trial and error method. Generallythe solvents such as toluene: acetone, benzene: ethyl acetate, n-Hexane: acetone solvents were used.

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixtures. Thin layer chromatography was performed on a sheet of glass, plastic, or aluminium foil, which was coated with a thin layer of adsorbent known as the stationary phase.

After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) was drawn up the plate via capillary action. Because different analyses the TLC plate at different rates, separation is achieved.

THIN LAYER CHROMATOGRAPHY

Procedure

Preparation of plate

20g of silica gel G was mixed with 30 ml of water and stirred well until fine slurry without clumps was obtained.

TLC spreader was adjusted to 0.3mm thickness and the aqueous slurry was poured and spread (A binding agent such as calcium sulphate can be incorporated with the slurry in order to facilitate the adhesion of the adsorbent to the plate).

The spreader was drawn from one end of the unit to the other end in an even rate over the glass plates (20X20cm)

- The plates were left to dry in air for sometime.
- The plates were kept horizontally in the oven at 105^{0}c for 1hr.
- The plates were removed from the oven and kept them in room temperature for 15min.
- Once the temperature of the plates was reduced to the room temperature it is ready for spotting.

Spotting

For the best separation and resolution the sample was dissolved in appropriate solvent and applied in the form of a band rather than a spot nearly2.5 cm from the edge, single spots tend to fail. The band usually spread over 2 to 2.5cm was applied drop wise with a micropipette or a micro syringe. The solvent need evaporation from time to allow more samples, to be added over a small area. A stream of nitrogen gas or drier was used for this purpose. A sample application is more convenient and has the advantage of even loading.

Preparation of Tank

A mixture of Butanol: Acetic acid: Water in the ratio 4:1:5 was used as a solvent (mobile phase). Poured the solvent in the depth of 1.5 cm and closed it with the lid, allowed to stand for one hour to ensure that the atmosphere with in the tank becomes saturated with solvent vapour (equilibrium). This helps the regular running of the solvent.

After the equilibrium the lid was removed and the TLC plate was placed vertically in the tank so that it stands in the solvent, care should be taken that there was about 1 cm distance between the level of solvent and spots (that was point of application of sample) on the plate. The lid was replaced (since the silica gel layer was the mobile phase across the layer occurs rapidly due to adsorption and capillary action with very little residence to flow).

The mobile phase was allowed to migrate across the spots toward the far end of the plate during which the test mixture was separated in to various components. (It was preferable to keep the system at a constant temperature when the development was occurring. The biggest advantages of TLC is the spread with which separation occurs. It has taken nearly 30min. The time depends upon the thickness of the immobile phase, choice of solvent and temperature.

- The irrigation solvent was allowed to run a distance of 12cm from the spot.
- The plates were removed from the tank and immediately dried at 60^0C for 30 min.

The separated amino acid spots were detected by spraying the plates with sulphuric acid. The sulphuric acid sprayed on the plates and heated at 110 ^{0}C.The compounds become and could be seen as brown spots. The distance traveled by each compound from the origin relative to the solvent front is defined as the Rf.The RF Value for the amino acid is calculated.

$$Rf = \frac{\text{Distance travelled by the substance from the origin}}{\text{Distance travelled by the solvent from the origin}}$$

Fourier-transform spectrometers

The Michelson interferometer

Radiation leaves the source and is split. Half is reflected to a stationary mirror and then back to the splitter. This radiation has travelled a fixed distance. The other half of the radiation from the source passes through the splitter and is reflected back by a movable mirror. Therefore, the path length of this beam is variable. The two reflected beams recombine at the splitter, and they interfere (e.g. for any one wavelength, interference will be constructive if the difference in path lengths is an exact multiple of the wavelength. If the difference in path lengths is half the wavelength then destructive interference will result). If the movable mirror moves away from the beam splitter at a constant speed, radiation reaching the detector goes through a steady sequence of maxima and minima as the interference alternates between constructive and destructive phases.If monochromatic IR radiation of frequency, f (ir) enters the interferometer, then the output frequency, fm can be found by; where v is the speed of mirror travel in mm/s.

Because all wavelengths emitted by the source are present, the interferogram is extremely complicated. The moving mirror must travel smoothly; a frictionless bearing is used with electromagnetic drive. The position of the mirror is measured by a laser shining on a corner of the mirror. A simple sine wave interference pattern is produced. Each peak indicates mirror travel of one half the wavelength of the laser. The accuracy of this measurement system means that the IR frequency scale is accurate and precise.

In the FT-IR instrument, the sample is placed between the output of the interferometer and the detector. The sample absorbs radiation of particular wavelengths. Therefore, the interferogram contains the spectrum of the source minus the spectrum of the sample. An interferogram of a reference (sample cell and solvent) is needed to obtain the spectrum of the sample. After an interferogram has been collected, a computer performs a Fast Fourier Transform, which results in a frequency domain trace (*i.e*

intensity vs. wavenumber) that we all know and love. The detector used in an FT-IR instrument must respond quickly because intensity changes are rapid (the moving mirror moves quickly). Pyroelectric detectors or liquid nitrogen cooled photon detectors must be used. Thermal detectors are too slow.

RESULTS AND DISCUSSION

Table1: Preliminary Phytochemical Screening of *Desmodiumgangeticum*

S.NO	TEST	Water	*Chloroform*	*Alcohol*
1	Alkaloids	+	+	+
2	Terpenoids	+	+	-
3	Steroids	_	+	-
4	Coumarins	-	+	+
5	Tannins	+	+	+
6	Flavonoids	_	+	-
7	Phenols	+	+	+
8	Volatile oils	_	-	-
9	Quinone	+	+	+
10	Saponin	+	+	+

Phytochemical Analysis

The preliminary phytochemical analysis was carried out for different extracts of *Desmodiumgangeticum.* The phytochemical analysis was carried out for three different extracts. The qualitative analysis of the ethanolic, chloroform and water extracts of *Desmodiumgangeticum.* revealed the presence of alkaloid, flavanoid, terpenoid,saponin, steroid, tannin and phenolic compounds, whereas steroids and valotile oil were absent.The Chloroform extract of *Desmodiumgangeticum*contain more compounds when compared to other solvents. The Chloroform extract of *Desmodiumgangeticum* showed of the presence of as saponin, alkaloids, steroids, terpenoids, coumarin,flavonoids, tannin, phenolic compound, and quinone were confirmed in suitable chemical tests. The aqueous extract of *Desmodiumgangeticum* contain alkaloid, terpinoid, tannin, saponin and phenolic compound. (Table1). Moreover, the highest yield was also observed in Chloroform extract and hence this was selected for further studies. (Table 1)

Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains.

Saponin is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyperchloles-trolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory, and weight loss etc. It is also known to have antimicrobial properties [Aiyelaagbe*et al.,*2009]. Plant steroids are known to be important for their cardiotonic, insecticidal and anti-microbial properties. They are also used in nutrition, herbal medicine, cosmetics and they are routinely used in medicine because of their profound biological activities. Tannin is reported to exhibit antiviral, antibacterial, anti-tumor activities. It was also reported that certain tannin are able to inhibit HIV replication selectivity and is also used as diuretic [Heslem*et al.,*1989].

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds . Many of these indigenous medicinal plants are used as spices. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999, 2001).

According to shen*et al.,* (1992), the flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. Farwuar (1996) included protection against free radicals, platelet aggregation, microbes, ulcers and hepatoxins. The phytochemical analysis revealed the presence of flavonoids in the herbal plants.

TLC Analysis

TLC analysis also suggested the presence of different kinds of phytochemicals in leaves extract. Thin layer chromatography was performed on plant extracts using different solvent systems Methanol:Water: Acetone (18:9:1).

TLC of plant extract in choloroform reports three spots for various phytochemicals. The reported spots are separated with enough space and having various Rf values showing the presence of at least three phytochemicals in chloroform extracts. In our study, the most suitable TLC system for analysis was shown to be Methanol:Water: Acetone (18:9:1) with the largest discriminating power. Three bands found in this method and its Rf values were 0.4,0.45 and 0.48.This values indicate the presence of phenolic compound.

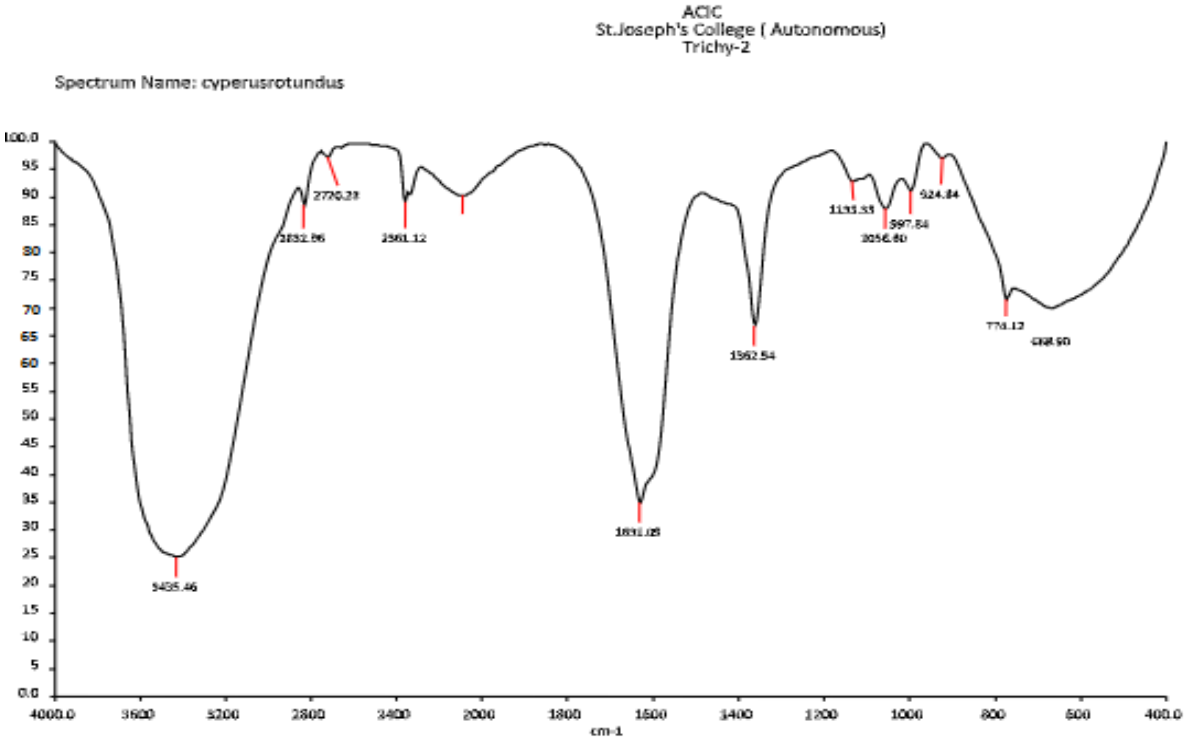

Figure 1: FT-IR Analysis of Chloroform extract of *Desmodiumgangeticum* leaves

S.NO	FREQUENCY RANGE	TYPE OF BOND	TYPE AND GROUP
1	3435.46	O–H stretch, H–bonded	alcohols, phenols

Table 2: FTIR analysis of chloroform extract of *Desmodiumgangeticum* leaves

2	2832.16	C–H stretch	Alkanes
3	2719.36	C-H-O, C–H stretch	Aldehydes
4	2361.12	C-H-O, C–H stretch	Aldehydes
5	2092.40	C≡ bond N stretch	Carboxyl
6	1631.09	N-H bend	Primaryamine
7	1133.33	C–H wag (–CH2X)	alkyl halides
8	1362.54	C-H rock	Alkanes
9	997.84	C-H bond	Alkens
10	924.84	o-H bend	Carboxylic acid

FT-IR Analysis

FT-IR measurement was carried out to identify the possible biomolecules in *Desmodiumgangeticum*. This spectrum shows lot of absorption bands indicates the presence of active functional groups in the *Desmodiumgangeticum*.. The intensity peaks are slightly increased for the period of 3435,2832,2719,2361 cm^{-1} as well as some intensity peaks decreased like 1362, 997, and 924 cm^{-1}.Fig: 1 shows the band at 3435 correspond to O-H Stretching vibrations of alcohol. The peak at 2719 represents to C-H in plane bend to alkenes. The peak at 997 corresponds to C-H, C-Br stretching vibrations to alkyl halides. The weak band at 1045 indicates C-O, C-N stretching vibrations and it corresponds to the presence of alcohols, carboxylic, acids, ethers, esters and aliphatic amines in the seed extract (Table.2 & Fig.1).

FT-IR spectra showing the presence of IR peaks assigned to polyphenols and also the existence of IR bands characteristic of amide I and amide II groups specific for proteins/enzymes suggest that flavonoids and proteins present in aqueous petal extracts of ornamental plants could be responsible controlling pathogen .**(K. Mallikarjuna. *et al.,* 2008)**

SUMMARY AND CONCLUSION

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use.Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs.The increase in prevalence of multiple drug resistance has slow down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from alternative Sources.

❖ The present study dealt with the Qualitative preliminary Phytochemical screening of *Desmodiumgangeticum(L)* was done.

❖ Qualitative preliminary Phytochemical analysis was performed in aqueous, chloroform and ethanolic extract of *Desmodiumgangeticum(L)*

❖ The chloroform extract contains more phytochemical constituentswhen compared to other extracts.

❖ The functional group in *Desmodiumgangeticum* also confirmed by FTIR spectroscopic technique.

❖ Some Phytoconstituent were separated in *Desmodiumgangeticum* extract by thin layer chromatography technique.

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