PHYTOCHEMICAL SCREENING GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF ALYSICARPUS MONILIFER L. (DC)

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ABSTRACT:

The present study was carried out to preliminary phytochemical evaluation and to characterize the bioactive constituents present is methanolic extracts of alysicarpus monlifier L .(DC) . (PAPILIONACEAE) using UV,FTIR and GC-MS(Preliminary qualitative chemical test for methanolic extract shows the presence of tannins, phenolic compounds,saponins, flavonoids, carbohydrate,phyto sterols.) The crude extracts were scanned in the wavelength ranging from 200-1100nm by using perkin elmer spectrophotomer and the characteristic peaks were detected. For GC-Ms analysis, while the mass spectra of the compounds found in the extract was matched by the NIST library. The FTIR spectrum shows may be the presence of aminoacid, phenol, alkanes, carboxylic acids, aliphatic amines and alkyl halides. The results of the GC-MS analysis, nine chemical constituents have been identified. The major chemical constituents were pentadecanoic acid 14-methyl,1-methyl ester(26.87%), tetradecanoic acid (11.08%), eruic acid (28.02%) and cyclic 3-1,2 ethane diyl acital (11.92%), The Extract was screened for antibacterial activity against staphylococcus aureas, pseudomonas, E-coli and basillus Alysicarpus monilifer showed potent antibacterial activity against gram negative and gram positive bacteria.

Key words: Alysicarpus monilifer, phytochemical GC-MS, UV, FTIR, Antibacterial activity.

INTRODUCTION:

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increase in demand in international trade because of very effective, cheaply available, supposely have no side effects and used as an alternative to allopathic medicines. Medicinal plants are believed to be much safer and proved elixir in the treatment of various ailments[1].

Alysicarpus monlifier L. (DC) (papilonaceae) commonly known as samervo (gujarati) or juhi ghas (hindi), is a turf forming legume and native to Africa, and asia. In india it is distributed through out the plains-madras,jammu,Bombay,punjob, Gujarat,madhyapradesh and uttar Pradesh. It is a prostate, procumbent or decumbent

perennialherb; stem of which is around 12-60 cm long, woody at the base. It is a branched; branches are terete clothed with covering trichomes. The herb is up to 50 cm in length and lairy when yound[(2,3)].

The plant was found to contain carbohydrates, phytosterols, saponins, phenolic compounds,tannic and flavonoids from the studies. Alysicarpus monlifier has been used in indigenous system of medicine as anti-inflammantory and in stomach-ache[4], an antidote to snake bite[5,6]. It is also used in skin diseases and as a diuretic[7,8]. The leaves are used in fever [9] and jaundice[10]. Within decade, there were a number of dramatic advances in analytical techniques including UV, FTIR, NMR, HPLC, and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals [11]. The aim of this study is to determine the bioactive compounds present in methanolic extracts of whole plant of alysicarpus monlifier with the aid of phytochemical evaluation, UV, FTIR, and GC-MS techniques.

MATERIALS AND METHODS:

COLLECTION OF PLANT MATERIALS:

The fresh plants of alysicarpus monlifier were collected from in the month of December 2015. The plants were collected from dindigul district.

The whole plants of alysicarpus monlifier were thoroughly washed with distilled water and kept in room temperature at 27^0c for two weeks. The dried samples were ground well to give particle size of 50-150nm[12-15].

PREPARATION OF EXTRACTS:
METHOD OF EXTRACTION:

Continuous hot percolatin (successive solvent extraction) process by using soxhelt apparatus and cold maceration method.

MATERIALS:

(I)	Soxhlet apparatus
(II)	Petroleum ether(60-80^{0}c)
(III)	Acetone (55-56^{0}c)
(IV)	Ethanol (75-78^{0}c)
(V)	Methanol (75-78^{0}c)
(VI)	Distilled water

EXTRACTION PROCEDURE:

The shade dried coarsely powered whole plant of alysicarpus monilifer NERS (50gm) was extracted with 250 ml of various solvents such as pet.ether, acetone, ethanol and methanol and distilled water in suitable temperature until the extraction was completed. After completion of extraction, the solvent was removed by distillation. It was then transferred to glass vials and kept at 4^0c before use.

PRELIMINARY SCRENING OF PHYTOCHEMICAL TEST:

Physiochemical screening of different extract from alysicarpus monilifer. The extracts were subjected to preliminary physiochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identify of a substance. The pharmacological actions of crude drugs were determined by the nature of their constituents the phyto constituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used. The petroleum ether extract. Acetone extract, ethanol, methanol and aqueous extract or alysicarpus monilifer was subjected to the following chemical tests used for the identification of various active constituents[16].

TESTS FOR ALKALOIDS[17]:

Dragendroff's test:

A fraction of the extracts were treated with dragondroff's reagent and observed for the formation of yellow colored precipitate, indicated the presence of alkaloids.

Wagner's test:

A fraction of the extracts were treated with wagner's reagent and observed for the formation of reddish brown precipitate, indicated the presence of alkaloids.

Mayer's test:

A fraction of the extracts were treated with mayer's reagent and observed for the formation of white precipitate or creamy colored precipitate, indicated the presence of alkaloids.

Hager's test:

A fraction of the extracts were treated with hager's reagent and observed for the formation of yellow precipitate, indicated the presence of alkaloids.

TEST FOR CARBOHYDRATES[17]:

Molisch's test, fehling's test, benedict's test, TESTS FOR GLYCOSIDES \ Legal's test borntrager's test were performed.

TESTS FOR PHYTOSTEROLS[(17)]

Libermann burchard test :

Mixed 3ml of the extracts were added with 3ml of acetic acid anhydride. It was heated and then cooled. Few drops of concentrated sulfuric acid were added. Appearance of blue color shows the presence of phytosterol.

Salkowski's test:

Dissolves the extracts in chloroform and equal volume of concentrate sulfuric acid was added. Formation of bluish red to cherry red color in chloroform layer and green fluorescence in the acid layer represented the steroid components present in the extract.)

TEST FOR FLAVONOIDS[18]:

Shinnodas test:

Small quantities of the extracts were dissolved in alcohol. To that some pieces of magnesium were added followed by concentrated hydrochloric acid was added drop wise and heated. Appearance of magenta color shows the presence of flavonoids.

Aqueous NaOH test:

Small quantities of various extracts were dissolved separately in aqueous sodium hydroxide. Appearance of yellow color indicates the presence of flavonoids.

Con.H_2SO_4 test:

To the small portion of each extract, concentrated sulfuric acid was added. Yellow orange colour was obtained shows the presence of flavonoids.

TEST FOR TANNINS AND PHENOLIC COMPOUNDS[19]:

Ferric chloride test :

1 ml of the extract were added with ferric chloride and observed for the formation of a dark blue or greenish black color indicated the presence of tannins and phenolic compounds

TEST FOR SAPONINS:

Foam test :

About 1ml of extracts were diluted separately with distilled water to 20ml and shaken in a graduated cylinder for 15 minutes. A 1% 1 cm layer of foam indicated the presence of saponins.

UV-VIS and FTIR Spectroscopic analysis:

The extracts were examined under visible and UV light for approximate analysis. For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through whatmann no.1 filter paper by using high pressure vacuum pump. The sample is diluted to 1;10 with the sample solvent. The extracts were scanned in the wavelength ranging from 200-1100nm using perkin elmer spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using perkin elmer spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each

and every analysis was repeated twice for the spectrum confirmation[20] .

GC-MS analysis:

2ul of them methanolic extract of alysicarpus monilifer was employed for GC/MS analysis was carried out on a GC clarus 500 perkin elmer system comprising a AOC-20^{0}C autosampler and gas chromatograph interfaced to a mass spectrophotometer (GC-MS) instrument employing the following conditions.cplumn elite-1 fused silica capillary column (30×0.25 mm 1D×1EM df.composed of 100% dimethyl poly siloxane) operating in electron impact mode of 70ev; helium (99.999%) was use carrier gas at a constant flow of 1ml/min and an injection volume of 0.5El was employed (split ratio of 10:1) Injector temperature 250^{0}C; ion-source temperature 280^{0}C. The oven temperature was programmes from 110^{0}C (isothermal for 2min) with an increase of 10^{0}C c/min, ending with a 9 min isothermal at 280^{0}C mass spectra were taken at 70 ev; a scan interval of 5 and fragments from 40 to 550 Da.

IDENTIFICATION OF COMPOUNDS:

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The defection employed the NIST ver 2.0 year 2005 (library). The compound prediction is based on Dr.jim duke of the agricultural research service/USDA. Interpretation of GC-MS was conducted using the database of NIST having more tha 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name molecular weight and structure of the components of the test materials were ascertained.

Antibacterial Assay:

The antimicrobial assay was performed by two methods viz., agar disc method (21) for aqueous extract and agar well diffusion method (22) for solvent extract. The molten Mueller Hinton agar (Hi Media) was inoculated with the 100 micro litre of the inoculums (1x10cfu) and poured into the sterile Petri plates (Hi-Media). For agar disc diffusion methods, the disc (0.7cm) (Hi-Media) was saturated with 100 micro litre of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork borer (0.85cm). 100 micro litre of the test compound was introduced into the well. The plates were incubated overnight at 37oc Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain controls were maintained where pure solvents used

instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented. The result was compared with the standard antimicrobics bacteria amecos.

RESULT& DISCUSSION

The phyto constituents were extracted by using different solvents of increasing polarity like petroleum ether, acetone , ethanol, methanol, and water. The results of whole plants of Alysicarpus monilifer were present in Table-1. In the above stated extracts, Most of the secondary metabolites were present in methanolic extracts.

They are carbohydrates , steroids, flavonoids, saponins, alkaloids,Glycosides phenolic compounds and tannins were presented. Flavonoids, as antioxidants may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may these reduce the occurrence of type 2 Diabetes [23.] Saponins are used in hypercholesterolemia , hyperglycemia, antioxidant, anticancer,anti inflammatory and weight loss etc.

TABLE NO.1 Preliminary Phytochemical investigation of different extracts of Alysicarpus Monilifer(L)DC

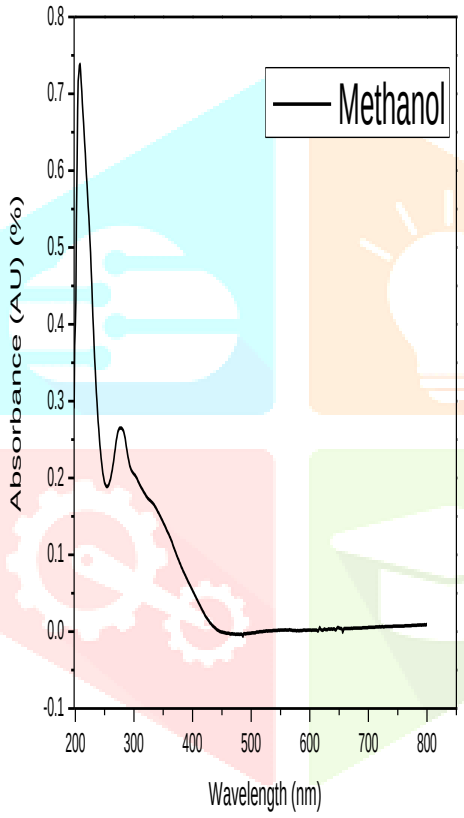
Phytochemicals	**Pet.ether Extract**	**Acetone Extract**	**Ethanol Extract**	**Methanol Extract**	**H_2O Extract**
Tannins	-	-	+	+	+
Phenolic compound	-	+	+	+	+
Saponins	-	-	-	-	+
Flavonoids	-	+	+	+	-
Carbohydrate	-	+	+	+	+
Steroids	+	+	-	+	_
Alkaloids	-	-	+	+	-
Phytosterol	-	_	+	+	-
Glycosides	-	+	+	+	+
Fixed oil and fats	-	-	-	-	-
Gums and mucilage	-	-	-	-	-

Note: + (Present), -(Absent)

UV absorption spectrum:

The qualitative UV spectrum profile of alysicarpus monilifer, methanolic

extract was selected at wavelength from 190-1100 nm due to sharpness of the peaks and proper basdines. The profile showed the peaks at 208 and 280 nm with the absorption of 0.738471 and 0.2638 respectively . (Figure-1 and table -2).



UV –Analysis of methanol extract of Alysicarpus monilifer

Table-2: UV –peak values of methanolic extracts of Alysicarpus monilifer

S.NO	Wavelength (nm)	Absorption peak
1	208	0.738471
2	280	0.26388

Functional group identification

The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alkanes, carboxylic acids, aminoacids, aliphatic amines and alkyl halides. (figure-2 and table 3)

Hence, the crude extracts subjected to UV and FTIR analysis is used for the identification of chemical constituents present in alysicarpus monilifer. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitivmethod [24] for detection of biomolecular composition.

Table-3: FTIR Peak values of methanolic extracts of Alysicarpus monilifer.

S.NO	Peak values	Functional group
1	3396.76	-NH2 Asy Stret

2	2974.33	-CH3 Antiasym Stret
3	2945.40	-CH2 Antiasym Stretching
4	2835.45	- CH2 Asym Stret
5	2524.90	-B-H Stretching
6	2044.61	C-C=-C-C=-C-CH
7	1653.05	-C=C- Stretching
8	1454.38	-CH3 Sym & Antisymmetrydeformation
9	1413.87	-CO- Stretching
10	1051.24	-C-C- Stretching
11	1026.52	-S=O Stret
12	1016.52	-C6H5 inplane deformations
13	879.57	-COC- Sym Stret
14	671.25	-CCl3 Sym Stret

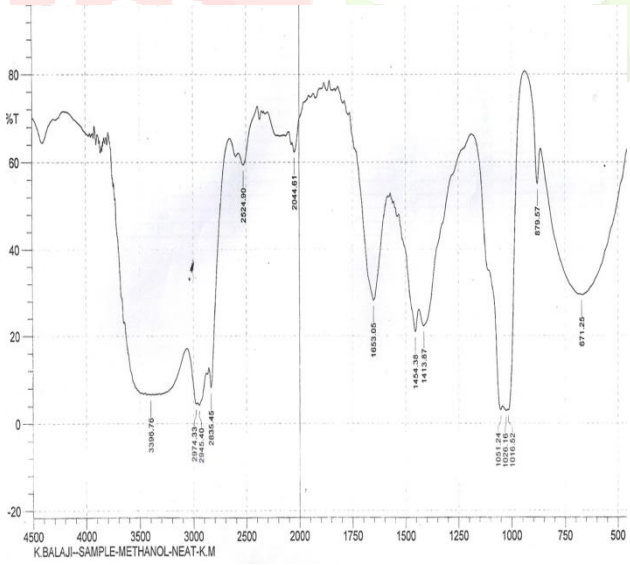


Figure- 2 FTIR analysis of crude methanol extract of AlysicarpusMonilifer

GC-MS analysis:

Nine compounds was identified in methanolic extracts of alysicarpus monilifer by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration % are presented in (fig-3 and table-4).

The major compounds were pentadecanoic acid 14-methyl , 1-methyl ester 26.87% tetra decanoic acid (11.08%), eruic acid (28.02%) and cyclic 3-1,2 ethanediyl acital (11.92%).

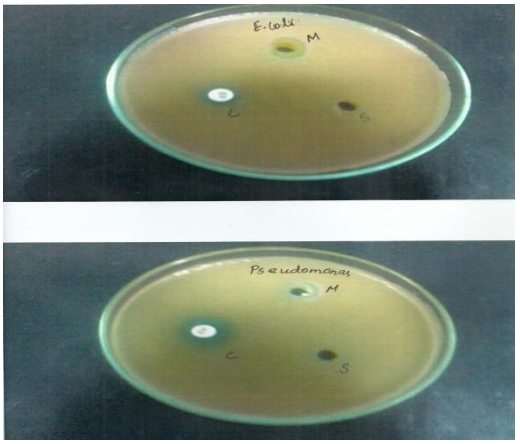
Sample	MethanolicExtract	C	SD
Staph aureus	15	R	20
Bacillus	10	R	18
E.coli	R	R	17
Pseudomonas	10	R	R

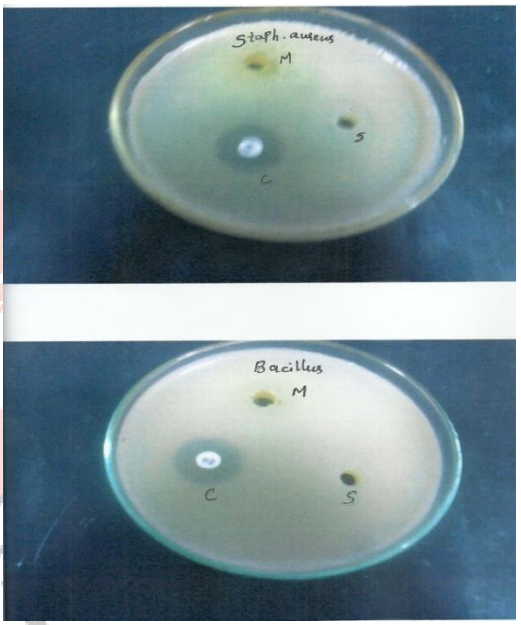
Figure -3 GC –MS Analysis of crude methanol extract of Alysiscarpus monilifer

Table-4: GC –MS Analysis of Chemical composition of the methanol extract of whole plant in Alysicarpus monilifer

S. No	R. Time	Area %	Compound Name
1	4.81	1.90	2 – tert- Butyl-5- proply 1.3-diaoxolan-4-one
2	10.23	26.87	Pentadecanoic acid, 14-methyl, 1-methyl ester
3	10.2	11.08	Tetradecanoic acid
4	11.01	28.02	Erucic acid
5	11.12	11.92	cyclic 3 – (1,2 ethanediyl acital)
6	11.87	7.09	Cis-13-Docosenoic acid
7	12.42	5.43	2-hydroxy – 1,3 propanediyl ester
8	13.06	4.52	Grape seed oil
9	13.16	3.16	Triarachine

Antibacterial activity of methanol extract of Alysicarpus monilifer;
Amikacin is standard disk for bacteria





The result of antibacterial activity of crude methanol extract of Alysicarpus Monilifer revealed that the highest activity against staph aureus and the lowest against Bacillus and pseudomonas. The antibacterial activity of methanolic extract of Alysicarpus Monilifer against the E. Coli is not significant.

So gram positive bacteria are more susceptible compare to the

Conclusion:

The present study on preliminary phytochemical evaluation of alysicarpus monilifer could be used as the diagnostic tool for the standardization of medicinal plant. The UV-VIS profile showed the peaks at 208 and 280nm respectively. The results of FTIR analysis confirmed the presence of

alkanes, carboxylic acids, aminoacids, aliphatic amines and alkyl halides. GC/MS results signified the presence of nine phytochemical constituents. The major compounds were pentadecanoic acid 14-methyl,1-methyl ester (26.87%), tetradecanoic acid (11.08%), eruic acid (28.02%) and cyclic 3-1,2 ethanediyl acital (11.92%)

Anti bacterial activity of methanolic extract of whole plant of Alysicarpus Monolifer showed the highest activity against Staph aureus than the Bacillus and Pseudomonas. So **gram positive bacteria** are more susceptible compare to **gram negative bacteria.** Methanolic extract shows good inhibitory effect against **Gram positive** and **Gram negative bacteria**

It could be concluded that alysicarpus monilifer contains various bioactive compounds. So it is recommended as lerbal alternative for various diseases including diabetic; cardiovascular diseases etc

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