Phytochemical Analysis and Antioxidant Activity of Leaf Extracts of *Alysicarpus vaginalis*

[1]Nandhu MR, [1]Abhirami VA, [1]Sukanya S, [1]Prabin John J, [2]PA Mary Helen
[1] Department of Biotechnology, Malankara Catholic College, Mariagiri Kaliakkavilai, 629153.
[2]Assistant Professor, Department of Biotechnology, Malankara Catholic College, Mariagiri Kaliakkavilai, 629153.

Abstract : Medicinal plants have impotent role in drug discovery. *Alysicarpus vaginalis* is one such medicinal plant from Fabaceae family. The present study was intended to analyse the presence of phytochemicals and antioxidant activity of aqueous, ethanol and benzene leaf extract of *Alysicarpus vaginalis*. In preliminary phytochemical analysis aqueous extract showed the presence of maximum compounds compared to ethanol and benzene extract. Benzene extract of *Alysicarpus vaginalis* showed highest antioxidant activity followed by ethanol and aqueous extracts. In GC-MS analysis 7 compounds were identified in which (R)-(+)-.Gamma.-caprolactone (37.40%) and 1,14-Tetradecanediol (26.98%) were identified as the major compound. The results of the present study showed that the leaf extracts of *Alysicarpus vaginalis* have significant antioxidant activity and phytocompounds.

Keywords - *Alysicarpus vaginalis*, phytochemical analysis, antioxidant activity, GC-MS analysis

1. INTRODUCTION

Plants have been used for medicinal purposes long before prehistoric period. Plants are the base of some of the traditional medicine systems such as Ayurveda, Unani etc. The world now faces a critical shortage of therapeutic drugs to treat disorders such as bacterial infections, cancer, diabetes, viral infections etc. This shortage had leaded the scientists to discover potential drugs from plants. Some drugs from Ayurveda approaches modern diseases, have already reached the market place [1].Plants are the storehouse of various bioactive compounds such as alkaloids, steroids, phenols etc. These are grouped as the secondary metabolites which are not directly involved in the growth, development or reproduction of plant.

Alysicarpus vaginalis (*A. vaginalis*) commonly known as alyceclover, one leaved clover (Malayalam: Nilaorila) is a flowering plant of Fabaceae family. They are more commonly found in continents such as Africa, Asia, Australia and the Americas. This species is an annual or perennial herb; different varieties may be either annual or perennial, and some behave as perennials in wet conditions but grows as annuals in dry regions [2, 3]. They are widely used for various diseases related with kidney, diuretics, leprosy and pulmonary problems [4]. *A. vaginalis* has important medicinal properties hence this work is mainly concerned on the study of primary phytochemicals and antioxidant property of different extracts of the plant *A. vaginalis*.

II. MATERIALS AND METHODS

2.1 Plant material

The leaves of *Alysicarpus vaginalis* were collected from Aralumoodu in Trivandrum district, Kerala (Figure 1).

Fig. 1: *Alysicarpus vaginalis*

2.2 Leaf extract

The fresh leaf sample was collected washed and shade dried. The dried leaves were then grinded to a fine powder using an electric grinder. About 25g of powdered sample was extracted with ethanol and benzene in a Soxhlet apparatus at a temperature not exceeding the boiling point of the solvents for 5 cycles. The collected extract was used for further studies.

An amount of 5gm of fresh leaves was weighed and grinded using mortar and pestle with 5ml of distilled water. Then the solution was kept for centrifugation at 5000rpm for 15 minutes and the supernatant was collected and filtered through Whatman number 1 filter paper and kept it under UV for 1 hour to prevent contamination and then stored at 5°C for further use.

2.3 Phytochemical screening

Phytochemical screening was carried out for all the three extracts as per the standard methods [5].

2.3.1. Test for flavonoids

Extracts were treated with two to three drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.3.2. Test for saponins

Extracts were diluted with 20 ml of distilled water and was shaken for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2.3.3. Test for tannins

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.3.4. Test for alkaloids

Extracts were dissolved individually in diluted hydrochloric acid and filtered using whatman filter paper

(a). **Mayer's test**

Filtrates were treated with Mayer's reagent. Formation of a yellow coloured precipitate indicates the presence of alkaloids.

(b). **Wagner's test**

Filtrates were treated with Wagner's reagent. Formation of brown/ reddish precipitate indicates the presence of alkaloids.

(c). **Dragendroff's test**

Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

2.3.5. Test for phenols.

Extracts were treated with 3-4 drops of freshly prepared ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

2.3.6. Test for amino acid

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

2.3.7. Test for proteins

The extracts were treated with few drops of concentrated nitric acid. Formation of yellow colour indicates the presence of proteins.

2.3.8. Test for diterpenes

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

2.4 Antioxidant activity of *A. vaginalis*:

In vitro evaluation of antioxidant activity by DPPH method

Radical scavenging activity of plant extracts against stable 2, 2diphenyl 2- picrylhydrazyl hydrate (DPPH) was determined by the slightly modified method [6]. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the concentration (1mg/1000µl). The solution of DPPH in methanol 60µM was prepared fresh daily before UV measurements. This solution (3.9ml) was mixed with 100µl of test solution at various concentrations (200, 400, 600, and 800µg).

The samples were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm on a UV visible light spectrophotometer. The experiment was carried out in triplicate. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank.

Radical scavenging activity was calculated by the following formula**.**

% Inhibition = (Absorbance of Control at 0 minute - Absorbance of Test) / Absorbance of Control at 15 minutes x 100

Where C= absorption of control sample (t= 0 min), C= absorption of control (t=15 min), T=absorption of test solution.

2.5 GC-MS Analysis

GC-MS analysis was carried out on a Shimadzu GC-MS (Model number QP2010S) instrument. The column used was Rxi-5Sil MS Capillary standard non-polar column measuring 30 m× 0.25 mm with a film thickness of 0.25 µm composed of 95% Dimethyl polysiloxane. Helium was used as carrier gas at a consistent flow of 1 ml/min and an injection volume of 0.5 µl is employed, injector temperature 260°C. The oven temperature is programmed from 70°C, with an increase of 10°C/ min, to 200°C then 5°C/ min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 550 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the NIST 11 and WILEY 8 Libraries. Measurement of peak areas and data processing were carried out by GC-MS Solutions software.

III. RESULTS

3.1 Phytochemical analysis

In the preliminary phytochemical screening ten compounds were tested for their presence or absence in three different extracts of *Alysicarpus vaginalis*. The results were summarized in table1.

Table: 1. Phytochemical Screening of *Alysicarpus vaginalis.*

Sl. No	Test conducted		Aqueous	Ethanol	Benzene
1	Flavonoids		+	+	-
2	Saponins		+	-	-
3	Tannins		+	+	-
4	Alkaloids	Mayer's test	-	-	-
		Wagner's test	+	-	-
		Dragendroff's test	+	-	-
5	Phenols		+	+	-
6	Amino acid		+	+	-
7	Proteins		+	-	+
8	Diterpenes		+	+	+

3.2 Antioxidant activity of *Alysicarpus vaginalis*:

In the present study the antioxidant activity of leaf extract (ethanol, aqueous, benzene) showed potential free radical scavenging activities. Benzene extract showed highest activity followed by ethanol extract and aqueous. The results were given in table 2 and figure 1.

Table: 2. Antioxidant activity of different extracts of *Alysicarpus vaginalis.*

SAMPLE NAME	MARKINGS	CONCENTRATION (µg)	OD AT 515 nm	% INHIBITION
ETHANOL (10mg/ml)	Control (at 0 mins)	-	0.466	-
	Control (after 15 mins)	-	0.454	-
	T1	200	0.418	10.5
	T2	400	0.380	18.5
	T3	600	0.340	27.75
	T4	800	0.310	34.36
AQUEOUS (10mg/ml)	Control (at 0 mins)	-	0.493	-
	Control (after 15 mins)	-	0.486	-
	T1	200	0.450	8.84
	T2	400	0.432	12.55
	T3	600	0.420	16.8
	T4	800	0.410	17.07
BENZENE (10mg/ml)	Control (at 0 mins)	-	0.491	-
	Control (after 15 mins)	-	0.484	-
	T1	200	0.402	18.3
	T2	400	0.332	32.85
	T3	600	0.310	37.39
	T4	800	0.292	41.11

Fig. 1: Shows antioxidant activity of different extracts of *Alysicarpus vaginalis*

Fig. 1(a). Ethanol extract

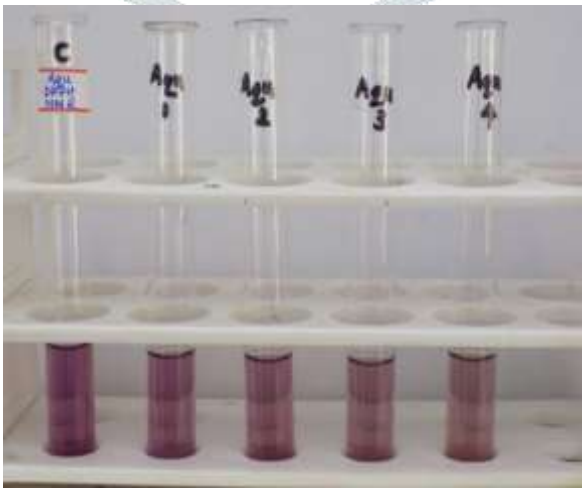

Fig. 1(b). Aqueous extract

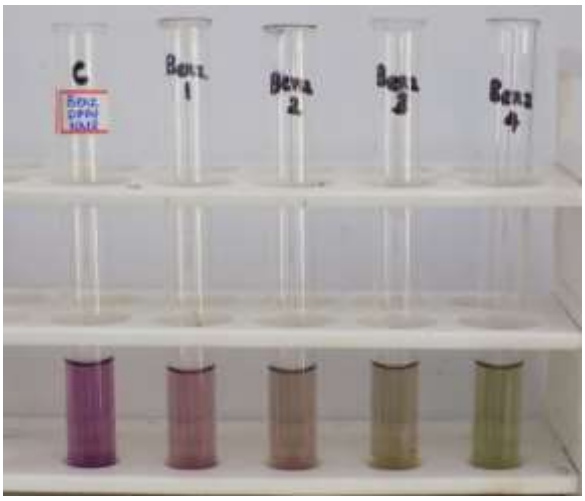

Fig. 1(c). Benzene extract

3.3 GC-MS Analysis

The bioactive compounds (secondary metabolites) present in the ethanol extract of leaf of *Alysicarpus .vaginalis* were identified by GC-MS analysis. Results were showed in table 3 and figure3. The most prevailing major compounds were (R)-(+)-.Gamma.-caprolactone (37.40 %) and 1,14-Tetradecanediol (26.98 %).

Table: 3. Separated bioactive compounds from the Ethanol leaf extract of *Alysicarpus vaginalis* identified by GC-MS**.**

SL.NO.	RETENTION TIME	COMPOUNDS	AREA %
1.	13.816	Ethanol, 2-butoxy-	1.10
2.	16.392	(R)-(+)-.Gamma.-caprolactone	37.40
3.	16.650	Butane, 1-propoxy-	17.63
4.	16.716	Hydroperoxide, 1,4-dioxan-2-yl	8.47
5.	16.764	1,14-Tetradecanediol	26.98
6.	22.522	Isophytol	6.71
7.	35.134	2,6,10-Dodecatrienoic acid,7,11-dimethyl-3-(trifluoromethyl)-.methyl ester, (Z,E)-	1.71

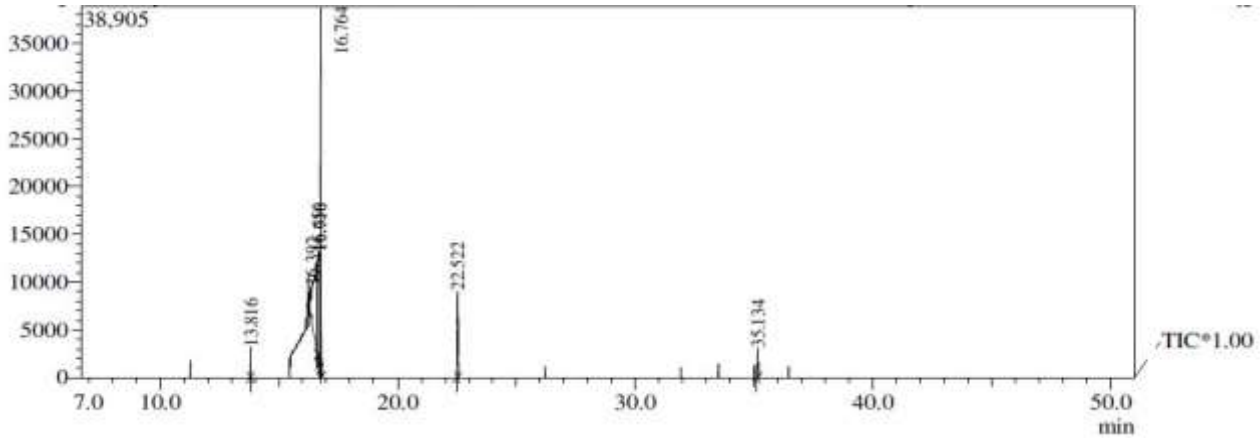

Fig. 3: Chromatogram of ethanol leaf extracts from *Alysicarpus vaginalis.*

IV. DISCUSSION

Medicine from plant sources have been in use in homeopathy, ayurvedic, allopathy and in traditional medicine since time immemorial. The aim of the present investigation is to evaluate the different extracts of *A. vaginalis* for their phytochemical composition and antioxidant activity. Alysicarpus vaginalis is used for the renal calculi treatment in traditional medicine [7]. The root of this plant is also used for diseases related with kidney and pulmonary problems [8]. Antioxidant and antibacterial properties of the ethanolic and aqueous extracts of *A. vaginalis* were reported [9]. Preliminary phytochemical screening of *A. vaginalis* showed the presence of polyphenols [10]. Because of the presence of these properties it is clear that plant possesses various medicinal properties.

In the present study the preliminary phytochemical analysis of the three extract revealed the presence of phenol, tannin, flavanoid, saponin, alkaloid, protein, amino acid, and diterpene. The aqueous extracts of *A. vaginalis* demonstrated maximum occurrence of phytoconstituents, followed by ethanol and benzene (Table 1). All the tested extracts showed the presence of diterpene. The phenol, tannin, flavanoid and amino acid showed their presence in both aqueous and ethanol extracts. The saponin is present only in the aqueous extract of *A. vaginalis*. The protein is present in both aqueous and benzene extract of *A. vaginalis*. Alkaloids are present only in aqueous extract of *A. vaginalis*. Alkaloids are used for medicinal purposes and they have positive as well as negative impact on human beings [11]. Studies show that polar fractions possess the major phytochemicals [12, 13].

In this study the scavenging activity of the three extract were done at different concentrations (200, 400, 600, and 800µg) (table 2). The study revealed that benzene extract showed 41.11% inhibition at 800µg concentration followed by ethanol (34.36%) and aqueous (17.07%) both at 800µg concentration. DPPH free radical scavenging method is an easy, rapid and sensitive way to screen the antioxidant activity of a specific compound or plant extracts [14].

The leaves of *A.vaginalis* were studied by GC-MS and 7 compounds were identified using this technique. Among these (R)-(+)-.Gamma.-caprolactone (37.40) and 1,14-Tetradecanediol (26.98) were identified as the major compounds. Gamma caprolactone is a commercial non-toxic compound used as a food flavor and as a fragrance in cosmetics and perfumes; Gamma caprolactone naturally occurs in numerous plant products [15]. Studies also shows that in hydroponic cultures of *S. tuberosum*, application of gamma- caprolactone stimulated the growth of NAHL- degrading R. erythropolis populations which are also able to use gamma- caprolactone as a carbon source [15]. The compound 1, 14 Tetradecanediol is known to possess an antimicrobial property [16].

V. CONCLUSION

This study concludes that A. *vaginalis* have interesting phytochemicals and antioxidant activity. The benzene extract of A. *vaginalis* showed significant antioxidant activity so it may also process anticancer activity. GC- MS analysis of ethanol extract of *A. vaginalis* showed the presence of various phytocompounds which could be used in modern agricultural practices to control diseases in plants and also in medicine to treat different ailments. Further studies are required to explore more about the medicinal as well as agricultural importance of the *A. vaginalis*.

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