

Preliminary Phytochemical Investigation and TLC Analysis of *P. Angulata* Fruit Extract

Raju Porika¹, Sateesh Poojari², Venkanna Lunavath³ and Estari Mamidala^{4*}

¹⁻⁴(Infectious Diseases & Metabolic Disorders Research Lab, Department Of Zoology,
Kakatiya University, Warangal, Andhra Pradesh, India)

Abstract: The present study is aimed to investigate the phytochemicals present in hexane, chloroform, ethyl acetate, acetone and methanolic extracts obtained from fruits of *Physalis angulata* plant and TLC profiling. The plant material was extracted with various solvents based on their polarity by the process of maceration method. Preliminary phytochemical analysis was performed by different qualitative methods. Preliminary phytochemical analysis of the extracts revealed the presence of carbohydrates, tannins, alkaloids, saponins and phenolic compounds. The solvent system selected for the best results of TLC was hexane and ethyl acetate of the ratio of 1:1, 1:2, 2:1 and 3:1. The study will provide referential information for the correct identification of the bioactive compounds and suitable solvent system for separation of those compounds from the fruit extract of *Physalis angulata*.

Keywords: Extract, *Physalis Angulata*, Phytochemical Analysis, Solvent, Rf And TLC

I. Introduction

The genus *physalis angulata* (locally called kakenge, winter cherry or chinies lanter (from solanaceae), an edible fruit and indigenous herb in india (Amini et al., 2004). *Physalis angulata*. Is widely distributed throughout tropical and subtropical regions of the world and its extracts or infusions have been used in many countries in popular medicine in the treatment of a variety of diseases such as malaria, asthma, hepatitis, dermatitis and rheumatism (Chiang HC et al.,1992, Lin YS et al., 1992, Soares MB et al.,2003.).*Physalis angulata* juice is considered to be sedative and depurative against rheumatism and earache. The leaves of the plant are sometimes used against inflammations of the bladder, spleen and liver. The whole plant cooked is recommended in baths for inflammatory processes, such as rheumatism (Lorenzi et al.,1982) and *Physalis angulata* have a broad spectrum of biological activities, including antibacterial, molluscicidal, antiprotozoal, anticancer, cytotoxic and immunomodulatory activities (Kastelein et al.,1990, Lee et al.,1991, Ismail et al.,2001). TLC is a simple, quick and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a unknown compound in a mixture when the Rf value of that compound is compared with the Rf value of a known compound. In the present study, we have attempted to investigate the phytochemical analysis of fruit extract.

II. Materials And Methods:

II.1. Plant Material And Extraction:

The fresh fruits of *Physalis angulata* were collected from Dharmasagar mandal, Warangal district, Andhra Pradesh, and the plant was authenticated by the professor V. Raju Department of Botany, Kakatiya University, Warangal. The shade dried *Physalis angulata* fruits were powdered mechanically and stored in an air tight container. The sequential extraction was carried out by maceration method. The solvents used were hexane, chloroform, ethyl acetate, acetone and methanol (non polar-polar). About 200 gm of powder was extracted with 800 ml of each solvent. The extract was concentrated to dryness under controlled temperature 40-50°C and pressure up to 1 atmosphere. The extract was preserved in refrigerator till further use.

II.2. Preliminary Phytochemical Analysis;

Preliminary phytochemical screening of the extracts for saponins, glycosides, alkaloids, tannins and flavonoids using standard phytochemical screening methods (Siddiqui et al.,1997, NR Earnsworth et al., 1974, Harborne JB et al.,2009)

II.2.1. Protocol For Qualitative Analysis:

The following tests have been done to detect the presence of the active chemical constituents. (Evans et al.,2003).

II.2.1.1. Alkaloids:

To detect the presence of alkaloids, a few drops of Mayer's reagent is added in solvent free extracts. Alkaloids solution produces cream coloured precipitate in presence of Mayer's reagent. Solvent free extract 50mg is stirred with few ml of dilute HCl and filtered take few ml of filtrate and add 1 or 2 ml of Hager's reagent. A prominent yellow precipitate indicates the presence of alkaloids.

II.2.1.2. Phenols:

To test the phenol phytochemical presence, in a test tube 1ml of extract and 2 ml of distilled water were added followed by few drops of 10% ferric chloride (FeCl_3). Appearance of blue or green colour indicates presence of phenols. The extract (50mg) is dissolved in distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

II.2.1.3. Test For The Carbohydrates:

Reducing sugars two methods were used to test for reducing sugars. First, the ethanol extract (1 ml) was added to 1ml of water and 20 drops of boiling Fehling's solution (a and b) in a test tube was added too. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars. Second, added to 2 ml of aqueous solution, 5-8 drops of boiling Fehling's solution. A red-brick precipitate showed the presence of reducing sugars. The extract (100mg) is dissolved in 5ml of water and filtered. To 0.5ml of filtrate, 0.5ml of Benedict's reagent is added. The mixture is heated on boiling water bath for 2 minutes. A characteristic coloured precipitate indicates the presence of sugars.

II.2.1.4. Cardiac Glycosides:

To test the cardiac glycoside phytochemicals presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride (FeCl_3) solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid (H_2SO_4). A brown ring of the interface indicates a de-oxy sugar characteristic of cardenolites. 50mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered, to 2ml of filtered 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia is added to it. Pink colour indicates the presence of glycosides.

II.2.1.5. Tannins:

To test the tannin phytochemical presence, in a test tube 1 ml of 5% ferric chloride added to solvent free extract. The presence of tannin is indicated by the formation of bluish black or greenish black precipitate.

II.2.1.6. Saponins:

To test the saponin phytochemicals presence in various extract, the extract was diluted with 20 ml distilled water and was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicates the presence of saponin.

II.3. TLC Analysis Of The Fractions:

Each of the aforesaid five extracts were, to begin with, checked by Thin Layer Chromatography (TLC) on analytical plates over silical gel. For each extract, four different solvent systems were used as developing systems. These were H-EA=1:1, 1:2, 2:1,3:1 where the standard abbreviations used are: H= hexane, EA = ethyl acetate. In each case, the spots were visualised by exposure of the plates to iodine vapour. Different bands were observed and corresponding R_f values are determined. R_f value of each spot was calculated = Distance travelled by the solute / Distance travelled by the solvent

III. Result:

III.1. Percentage Of Yield Extract:

The fruit extracts of *physalis angulata* were weighed. The amount obtained from hexane, chloroform, ethyl acetate, acetone and methanol extracts are 7.3gm, 6.2gm, 1.5gm, 1.3gm and 5.7gm respectively. The percentage of yield was calculated and presented in table-1.

Table-1: the percentage of yield of different extracts of *physalis angulata* fruit

S.no.	Solvent	Color of extract	Yield of the extract(in gm)	Percentage of yield(%w/w)
1	Hexane	Brown	7.3	3.65
2	Chloroform	Brown	6.2	3.1
3	Ethyl acetate	Brown	1.5	0.78
4	Acetone	Brown	1.3	0.65
5	Methanol	Brown	5.7	2.85

III.2. Preliminary Phytochemical Screening:

The present study carried out in the *Physalis angulata* revealed the presence of medicinal active constituents. The phytochemical active compounds of *Physalis angulata* were qualitatively analyzed for the fruits and the results are presented in table-2. In this screening process alkaloids, phenols, tannins, saponins, glycosides and carbohydrates shows different types of results in different solvent extracts. Among these phytochemical screening, alkaloids and carbohydrates were present in all solvent extracts, where as tannins present in all extracts except ethyl acetate, phenols were present in only hexane, chloroform, ethyl acetate except acetone and methanol. Saponins were absent in all solvent extracts.

Table-2: phytochemical studies of *physalis angulata*

+ Indicates the presence of phytochemical

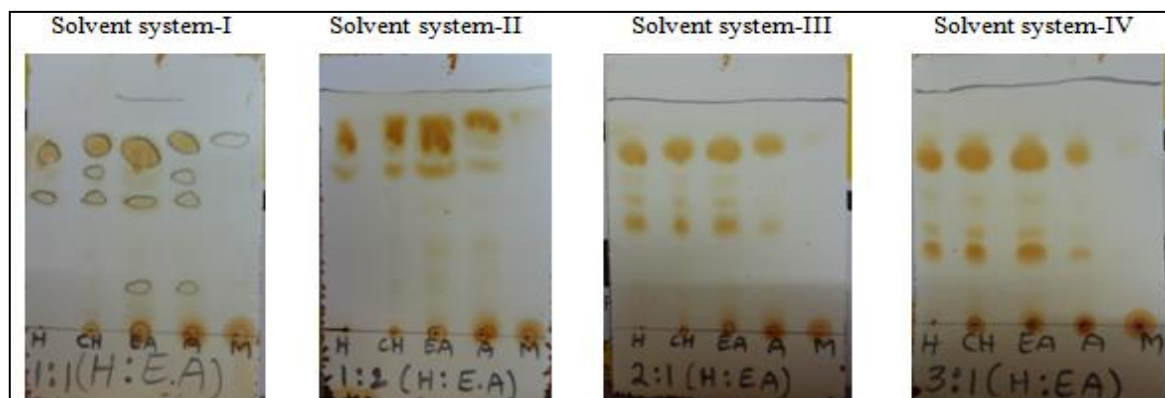
-Indicates the absence of phytochemical

Phytochemicals	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Alkaloids	+	+	+	+	+
Phenols	+	+	+	-	-
Tannins	+	+	-	+	+
Saponins	-	-	-	-	-
Glycosides	+	+	+	-	+
Carbohydrates	+	+	+	+	+

III.3. TLC Analysis:

In solvent system-i (1:1,H:EA), hexane extract shows 2 spots with Rf values 0.77 and 0.55, chloroform extract shows 3 spots with Rf values 0.82,0.67 and 0.55, ethyl acetate extract shows 3 spots with Rf values 0.80, 0.55 and 0.22, acetone extract shows 4 spots with Rf values 0.85, 0.70, 0.55 and 0.20, methanol extract shows 1 spot with Rf value 0.85.

Figure-2. TLC plate with: Solvent system-I: Hexane:Ethyl acetate (1:1), Solvent system-II: Hexane:Ethyl acetate (1:2), Solvent system-III:, Hexane: Ethyl acetate (2:1), Solvent system-IV: Hexane:Ethyl acetate (3:1)



In solvent system-ii (1:2,H:EA), hexane extract shows 2 spots with Rf values 0.82 and 0.65, chloroform extract shows 3 spots with Rf values 0.87,0.63 and 0.21, ethyl acetate extract shows 3 spots with Rf values 0.85, 0.65 and 0.36, acetone extract shows 3 spots with Rf values 0.87, 0.63 and 0.36, methanol extract shows 1 spot with Rf value 0.85.

In solvent system-iii (2:1,H:EA), hexane extract shows 3 spots with Rf values 0.84, 0.61 and 0.48, the chloroform extract shows 3 spots with Rf values 0.84,0.58 and 0.46, ethyl acetate extract shows 3 spots with Rf values 0.84, 0.58 and 0.46, acetone extract shows 2 spots with Rf values 0.87 and 0.46, methanol extract shows 1 spot with Rf value 0.87.

In solvent system-iv (3:1,H:EA), hexane extract shows 4 spots with Rf values 0.74, 0.56, 0.41 and 0.35, chloroform extract shows 4 spots with Rf values 0.74,0.53, 0.41 and 0.35, ethyl acetate extract shows 4 spots with Rf values 0.76, 0.53, 0.41 and 0.33, acetone extract shows 2 spots with Rf values 0.74 and 0.30 methanol extract shows 1 spot with Rf value 0.79.(fig-2.)

Table-3: (TLC profiling) showing R_f values of fruit extracts of *Physalis angulata* in various solvent systems

S. No	Solvent System Hexane: Ethyl Acetate	Extract	No. Of Spots	F _r Value I	F _r Value II	F _r Value III	F _r Value IV
1	1:1	H	2	0.77	0.55	-	-
		C	3	0.82	0.67	0.55	-
		EA	3	0.80	0.55	0.22	-
		A	4	0.85	0.70	0.55	0.20
		M	1	0.85	-	-	-
2	1:2	H	2	0.82	0.65	-	-
		C	3	0.87	0.63	0.21	-
		EA	3	0.85	0.65	0.36	-
		A	3	0.87	0.63	0.36	-
		M	1	0.85	-	-	-
3	2:1	H	3	0.84	0.61	0.48	-
		C	3	0.84	0.58	0.46	-
		EA	3	0.84	0.58	0.46	-
		A	2	0.87	0.46	-	-
		M	1	0.87	-	-	-
4	3:1	H	4	0.74	0.56	0.41	0.35
		C	4	0.74	0.53	0.41	0.35
		EA	4	0.76	0.53	0.41	0.33
		A	2	0.74	0.30	-	-
		M	1	0.79	-	-	-

H=hexane, C=chloroform, EA=ethyl acetate, A=acetone, M=methanol

IV. Discussion;

Recently, much attention has been directed towards extracts and biologically active compounds isolated from popular plant species. The use of medicinal plant plays a vital role covering the health needs in developing countries, and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective bacteria. (Coelho de Souza G *et al.*, 2004). The current investigation reports reveal that fruit extracts of *Physalis angulata* possessed numerous phytochemicals that are indirectly or directly attribute biological activity of extracts. However, all the extracts showed negative response for test carried out for the detection of saponin presence. Among the various extracts, acetone and ethyl acetate also showed negative response for the test carried out for the identification of tannins and phenols and thus elucidates its poor or no biological activity. Whereas, methanol extract noticed the presence of almost all phytochemicals except saponin content. Thus, immense activity of methanol extract is might probably from the base of these phytochemicals.

TLC results indicates all the mobile phases are suitable to separate the compounds with good resolution which are present in those extracts to Column chromatography except 2:1(H:EA).

V. Conclusion:

In this study, the fruit of *Physalis angulata* have a various chemical groups in their chemical composition. It revealed some differences in the constituents of various solvent extracts. All the five extracts tested positive except saponins, which gave the important therapeutic and medicinal value to fruit extracts. It would be interesting to purify and identify different molecules and tested it in vitro and in vivo by exploiting on animal models, to specify this therapeutic application.

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