International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 4 | OCT-DEC | 2018 | 394-401
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

EVALUATION OF METHANOLIC EXTRACT OF *PHYSALIS MINIMA* FRUITS FOR IMMUNOMODULATORY ACTIVITY

K. Sunitha[1]*, M. Nagulu[2] and K. Srisailam[3]

[1, 3]- University College of Pharmaceutical sciences, Satavahana University, Karimnagar, Telangana, India- 505001
[2]- Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India-508004

***Corresponding Author Email: kailasasuni@gmail.com**

ABSTRACT

Traditional plants are used to treat several ailments. They are rich source of variety of phytoconstituents. The present phytochemical studies have addressed extracting, isolating and identifying bioactive compounds of plants. In the present study, Physalis minima unripe fruits were extracted with methanol and screened for active group of chemical constituents by different analytical methods. The literature survey revealed that there is no evaluation of immunomodulatory activity of the fruits. So, the present work was aimed to evaluate the immunomodulatory activity of methanolic extract of unripe fruits of Physalis minima. From research studies in the past and present HPLC and LC-MS data, it can be assumed that the extract possess steroidal alkaloids, which are responsible for immunostimulant properties.

KEY WORDS

Phytochemical, methanolic extract, Physalis minima, HPLC, LC-MS, Immunomodulatory activity

INTRODUCTION

Immunomodulatory activity means the biological or pharmacological effects of compounds on humoral or cellular aspects of the immune response[1]. For maintaining a disease-free state, modulation of immune response either through stimulation or suppression is required[2]. There are certain agents, which are apart from being specifically stimulatory or suppressive, have been shown to possess activity to modulate pathophysiological processes and are hence addressed as immunomodulatory agents[3].

They are also known to be biological response modifiers like haemopoietic drugs. Drugs may modulate immune mechanism by either suppressing or by stimulating in any of the following ways: by antigen recognition and phagocytosis, by lymphocyte proliferation/differentiation, by synthesis of antibodies, by antigen-antibody interaction, by release of mediators due to immune response, modification of target tissue response[4,5].

Physalis minima is a perennial herb belonging to the family Solanaceae, commonly known as pygmy ground cherry, wild cape gooseberry, native gooseberry. It is pantropical annual herb possess cream to yellowish flower followed by edible yellowish fruit encapsulated in papery cover which turns straw brown on maturity[6, 7]. The results of the preliminary phytochemical analyses in the chloroform, diethyl ether, ethanol, ethyl acetate and methanol extracts of stem, leaves and unripe fruits showed presence of Alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins and terpenoids. Reducing sugars were unable to be separated in all the solvent extracts of P.minima. Amount of phenols eluted by the organic solvents was very low in all the plant parts[8].

The past studies reported that the plant possess diuretic activity, anti-inflammatory, analgesic, antipyretic, antibacterial, antidiabetic activities[9-12].

The literature survey revealed that there is no detailed study of chemical constituents using analytical methods such as HPLC, I.R., and LC-MS. So, the present work was aimed to study the detailed chemistry of active principles present in the methanolic extract of unripe fruits of *Physalis minima*.

MATERIALS AND METHODS

Collection of Plant Material

The unripe fruits of *Physalis minima* were collected from the fields of Jammikunta, Karimnagar, Telangana, India. The plant parts were authenticated and deposited at the herbarium of University College of Pharmaceutical Sciences, Satavahana University, Karimnagar, Telangana, India.

Preparation of the extract

The unripe fruits of *Physalis minima* (2.0kg) were kept for maceration with methanol for seven days. The extracts were concentrated in desiccators[13].

Chemicals

All the chemicals used for the investigation were of analytical grade.

Drugs

In the present study Levamisole was used as an immunostimulating agent[14].

Antigenic material

All the above groups mice were antigenically challenged with SRBC (0.5x109cells/ml/100 g) on the 5th day intraperitoneally[14].

Detection of phytoconstituents

The extract was tested for phytoconstituents by preliminary tests, separated the constituents by HPLC and identified molecular weight by LC-MS.

Screening of immunomodulatory activity

Methods

- Carbon clearance test
- Humoral antibody titre
- Delayed type hypersensitivity

METHOD OF EVALUATION

Detection of Phytoconstituents

The extract was tested for the presence of Carbohydrates, Tannins, Flavonoids, Alkaloids, Anthocyanin and Betacyanin, Glycosides, Proteins, Steroids and Phytosterols, Phenols.

Chromatography

a. Thin-layer chromatography (TLC)

TLC was used for detecting the class of compounds present in the sample[15, 16].

b. Preparative high-performance liquid chromatography

This technique was used to identify the specific constituent present in the sample, which was idsolated by column chromatography[17].

HPLC conditions

Column: Hypersil BDS-C18 (150X4.6mm, 5µ)
Mobilephase: A: 0.1% TFA in Water (50%)
B: 0.1% TFA in ACN (50%)
Flowrate: 1.0 ml/min
Column temp: 35°C
Run time: 40min
Programme (Isocratic)
Diluent:MeOH
Sample Preparation: 1.0 mg/mL in diluent
Vail: 96
Injection Volume: 10 uL

b. Liquid Chromatography-Mass Spectroscopy (LC-MS)

Method: D:\Methods\General-5.lcm
Method Parameters: Column: Hypersil BD
S C-18 150 X 4.6 mm, 5 µm
Mobile Phase:A:Acetonitrile
Mobile Phase: B: 5mM Ammonium acetate in water
Gradient Time: -0.01 10.0 30.0
B%: - 95 10 10
Flow Rate: 1.0 mL/min
Sample Preparation: in MeOH: ACN
Note: Filtered sample was taken

Screening of immunomodulatory activity

Carbon clearance test

Swiss albino mice were divided into five groups which were administered drug for 5 days orally. On the last day, mice were injected with 0.1mlIndian ink via the tail vein. Blood samples were withdrawn at 0min and 15min. A 50µL blood sample was mixed with 4ml, 0.1% Sodium carbonate solution and the absorbance of this solution was determined at 660nm. The phagocytic index K was calculated using the following equation:

K= (Log OD1-Log OD2/15

where OD1 and OD2 are the optical densities at 0 and 15min respectively.

Delayed type hypersensitivity

Cell-mediated immunity (CMI) involves effector mechanisms carried out by T-lymphocytes and their

products (lymphokines). The cell mediated immune response was assessed by DTH reaction, i.e. Footpad reaction[18-20].

Humoral antibody titre

The animals were immunized by injecting 0.1ml of SRBCs suspension, containing 1×10^8 cells, intraperitoneal on day 0. Blood samples were collected in micro centrifuge tubes from individual animal by retro orbital puncture on day 7. Briefly, equal volumes of individual serum samples of each group were pooled. To serial two-fold dilutions of pooled serum samples made in 25µl volume of normal saline, in U-bottomed micro titration plates were added 25µl of freshly prepared 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37ºc for 2h and examined visually for agglutination. The reciprocal of the highest dilution of the test serum causing visible haemagglutination was taken as the antibody titre

RESULTS AND DISCUSSIONS

The unripe fruits of *Physalis minima* were extracted with methanol and screened for active group of chemical constituents using primary phytochemical tests. The extract showed positive results for alkaloids (Table 1-2).

Table 1: Percentage yield of various extract of unripe fruits of *Physalis minima*

Sl. No	Extract	Yield (%w/w)	Extract colour
1	Methanol soluble	0.20	brown

Table 2: Detection of Phytoconstituents

Dried leaves extracted with Organic solvents	Alkaloids (Maeyer's test)	Glycosides (borntragers' test)	Carbohydrates (Molisch test)	Steroids Liebermann-Buchard test	Phytosterols	Tannins (FeCl3 test)	Phenols (FeCl3 test)	Saponins (Foam test)	Flavonoids (H2So4 test)	Proteins (Ninhydrin test)	anthocyanins	Betacyanins
Methanol	+	+	+	+	-	+	+	-	-	-	+	-

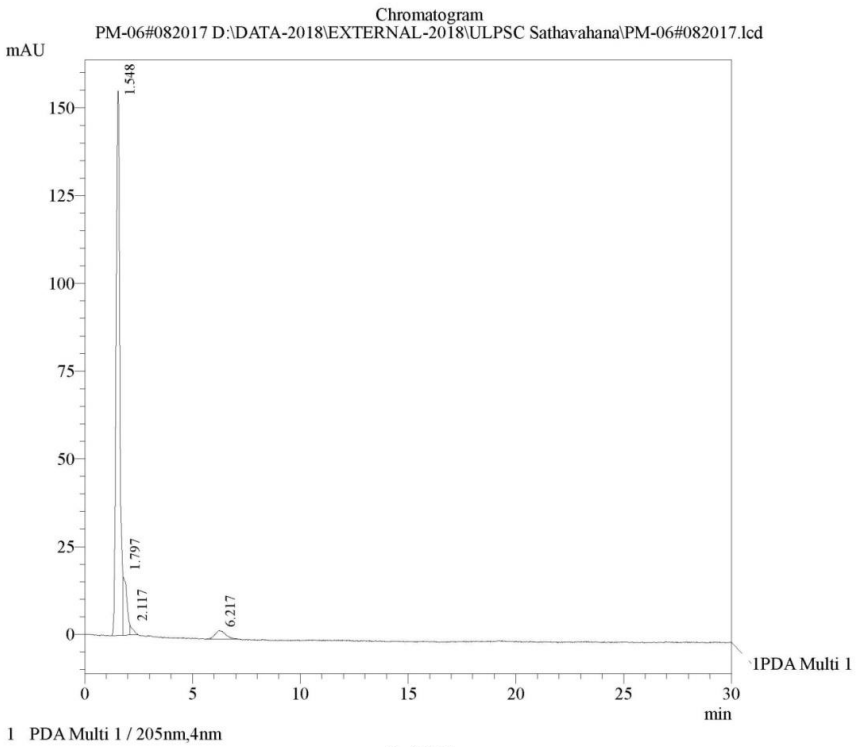
+ indicates present; - indicates absent

Thin-layer chromatography (TLC)

TLC analysis

- Chloroform: Ethanol was used in the ratio of 9:1, orange coloured spot was observed on TLC[21, 22] (Fig.1)

Fig.1

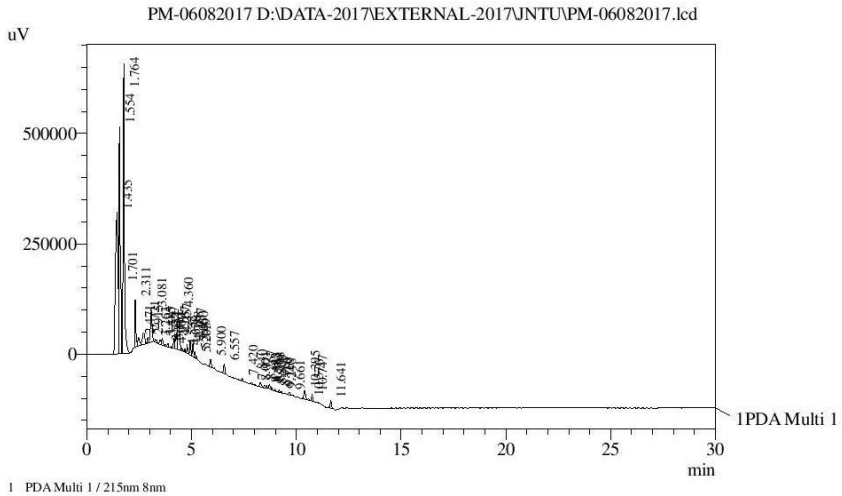


1 PDA Multi 1 / 205nm,4nm

PeakTable

PDA Ch1 205nm

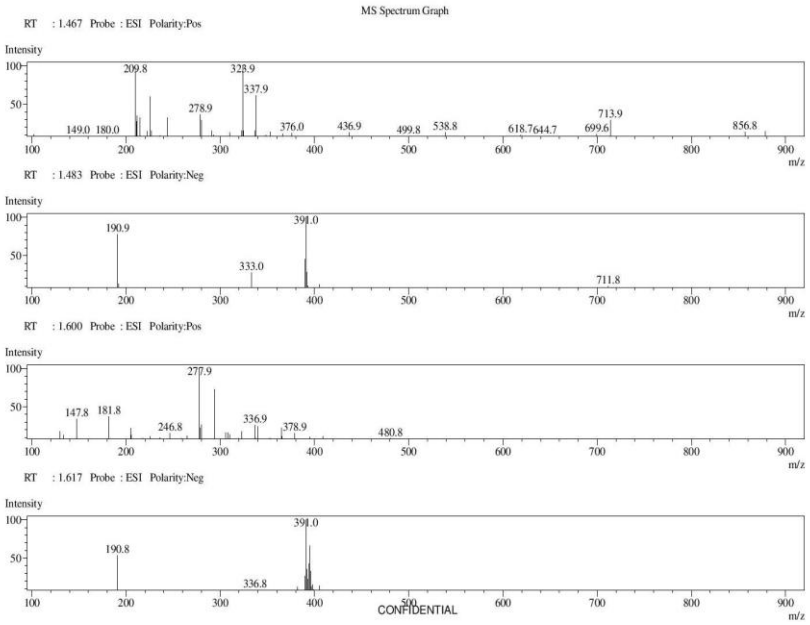
Peak#	Ret. Time	Area	Relative Retention Time	Area %
1	1.548	1916775	1.000	86.178
2	1.797	196535	1.161	8.836
3	2.117	24826	1.368	1.116
4	6.217	86070	4.017	3.870
Total		2224206		100.000

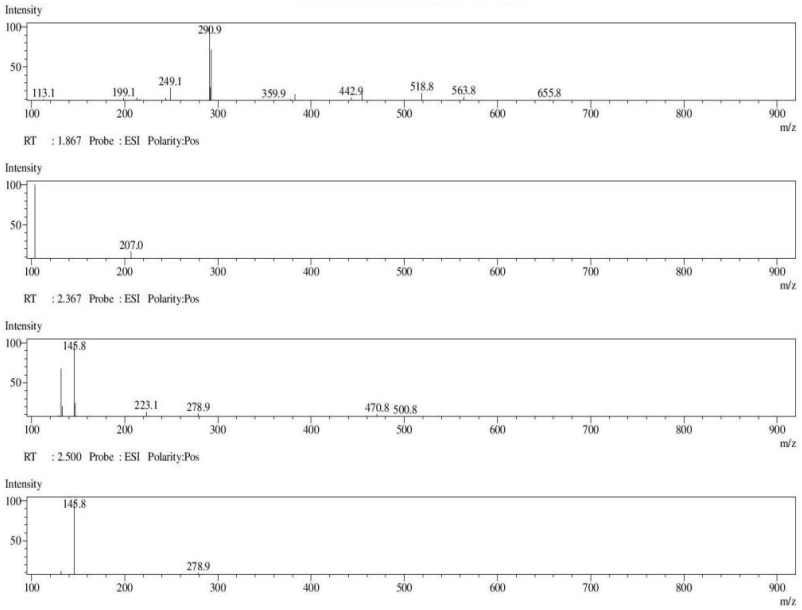


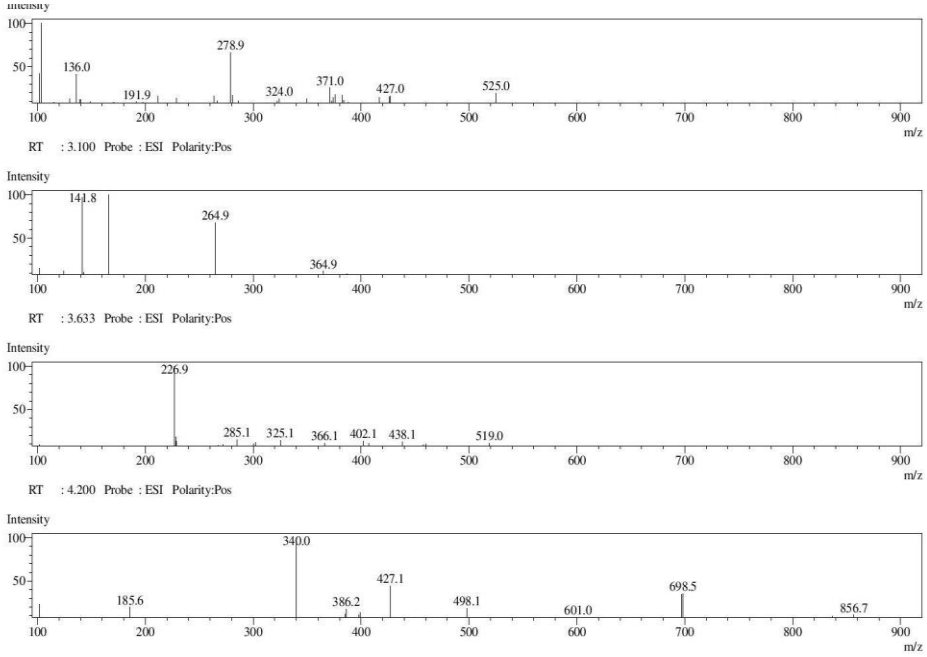
1 PDA Multi 1 / 215nm 8nm

PDA Ch1 215nm 8nm

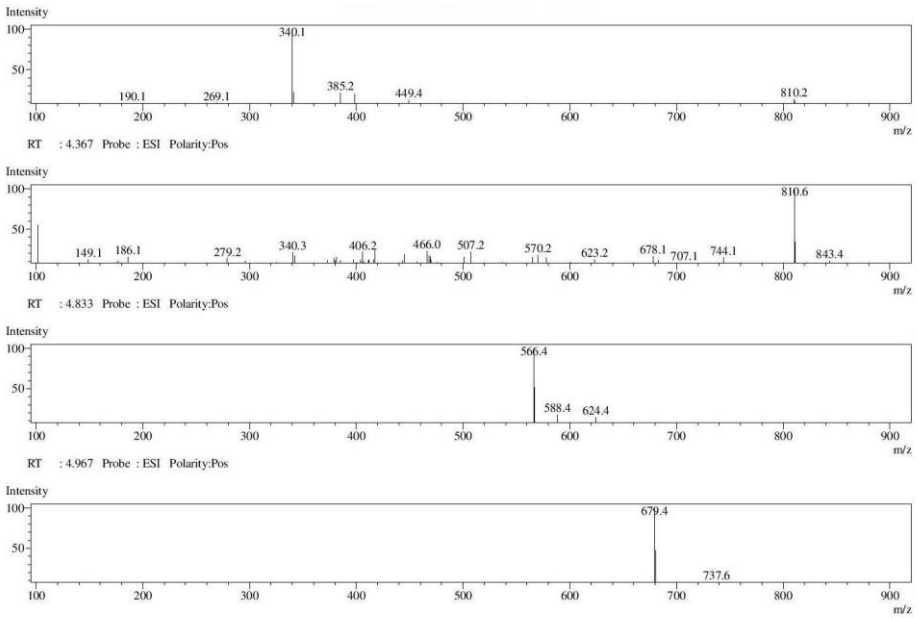
Peak#	Ret. Time	Area	Area %	Relative Retention Time
1	1.435	2655399	23.570	1.000
2	1.554	2375722	21.088	1.083
3	1.701	480391	4.264	1.185
4	1.764	2545965	22.599	1.229
5	2.311	332433	2.951	1.610
6	2.471	125149	1.111	1.721
7	2.721	210908	1.872	1.896
8	2.915	53934	0.479	2.031
9	3.081	373673	3.317	2.147
10	3.263	47251	0.419	2.274
11	3.485	55350	0.491	2.428
12	3.597	75646	0.671	2.506
13	3.757	19288	0.171	2.618


MS Spectrum Graph
RT : 1.467 Probe : ESI Polarity:Pos
Intensity
209.8
328.9
337.9
278.9
149.0 180.0
376.0
436.9
499.8
538.8
618.7644.7
699.6
713.9
856.8
m/z
RT : 1.483 Probe : ESI Polarity:Neg
Intensity
190.9
391.0
333.0
711.8
m/z
RT : 1.600 Probe : ESI Polarity:Pos
Intensity
277.9
147.8
181.8
246.8
336.9
378.9
480.8
m/z
RT : 1.617 Probe : ESI Polarity:Neg
Intensity
391.0
190.8
336.8
m/z
CONFIDENTIAL


Intensity
290.9
113.1
199.1
249.1
359.9
442.9
518.8
563.8
655.8
m/z
RT : 1.867 Probe : ESI Polarity:Pos
Intensity
207.0
m/z
RT : 2.367 Probe : ESI Polarity:Pos
Intensity
145.8
223.1
278.9
470.8
500.8
m/z
RT : 2.500 Probe : ESI Polarity:Pos
Intensity
145.8
278.9
m/z



Peak#	Ret. Time	Area	Area %	Relative Retention Time
14	3.861	31286	0.278	2.690
15	4.009	10112	0.090	2.793
16	4.167	103293	0.917	2.903
17	4.257	135434	1.202	2.966
18	4.360	464664	4.125	3.038
19	4.532	20602	0.183	3.157
20	4.670	24085	0.214	3.254
21	4.788	69159	0.614	3.336
22	4.937	121544	1.079	3.440
23	5.060	111585	0.990	3.525
24	5.149	50164	0.445	3.588
25	5.205	16192	0.144	3.627
26	5.900	88369	0.784	4.111
27	6.557	90897	0.807	4.568
28	7.420	23147	0.205	5.169
29	7.840	19901	0.177	5.462
30	8.001	7385	0.066	5.575
31	8.267	56281	0.500	5.760
32	8.483	27751	0.246	5.910
33	8.583	24184	0.215	5.980
34	8.708	47690	0.423	6.067
35	8.800	14950	0.133	6.131
36	8.964	20301	0.180	6.246
37	9.169	23346	0.207	6.388
38	9.248	14508	0.129	6.443
39	9.661	43738	0.388	6.731
40	10.395	107518	0.954	7.242
41	10.576	10602	0.094	7.369
42	10.747	60585	0.538	7.488
43	11.641	75484	0.670	8.111
Total		11265869	100.000	



HPLC analysis

Results of HPLC analysis of methanolic crude extract unripe fruits of *Physalis minima*, at 330 nm, shows presence of active constituents as evidenced by the chromatogram obtained at retention time 1.548, 1.797, 2.117, 6.217 with corresponding retention area 1916775, 196535, 24826, 86070, 2224206.

LCMS analysis

HPLC coupled with different detection methods e.g. UV, MS provided a preliminary information about the content and nature of constituents found in the active extracts i.e., steroidal alkaloids.

By selective ion monitoring in LC/MS or even LC/MSMS, it is possible to achieve the detection of specific target molecules - those, for example, which have already been found to exhibit a particular activity. The recent introduction of other hyphenated techniques such as LC/NMR will render the on-line structure determination of metabolites even more accurate and rapid[23].

Screening of immunomodulatory activity

The animals were screened using the haemagglutinating antibody titre to assess humoral immune response and Carbon clearance test to assess scavenging activity. The animals were also evaluated for delayed type hypersensitivity by the difference between the pre and post challenge footpad thickness. They have shown significant immunostimulant properties i.e., immunodulatory activity for all the methods used. The data were analyzed using statistical methods and compared to that of the standard drug, obtained values at a dose of 200mg/kg body weight (Table 3).

Table 3: Effect of methanolic extract of unripe fruits of *Physalis minima* by Carbon Clearance test, Humoral Antibody (HA) Titre and Delayed Type Hypersensitivity (DTH) response

Treatment dose	Carbon Clearance test	DTH response	HA Titre
Control	0.065± 0.2	09.00± 0.011	3.20±0.51
Std (Levamisole)	0.069± 0.1	09.84± 0.013	3.36±0.54
TME (200mg)	0.073±0.1	12.09± 0.020	5.05±0.22
TME (400mg)	0.075±0.2	12.88± 0.032	7.38±0.45
TME (600mg)	0.079±0.2	14.86± 0.036	10.90±0.51

CONCLUSION

The HPLC and LC-MS of the methanolic crude extract of unripe fruits of *Physalis minima* showed the presence of active constituents i.e., steroidal alkaloids. Based on the past literature survey and present study results, it can be assumed that the extract possesses steroidal

alkaloids, which are responsible for stimulant properties. So, it has been concluded that the extract of unripe fruits of *Physalis minima* could be used as a drug to strengthen immunity to fight against various infections.

ACKNOWLEDGEMENTS

I would like to thank Dr. E.N. Murthy for his contribution in authenticating the plant parts.

REFERENCES

1. Wagner H, Search for plant derived natural products with immunomodulatory activity: recent advances; Pure and Applied Chemistry; 1990; 62(7): 1217-1222.
2. Ghule BV, Murugananthan G and Yeole PG, Immunostimulant effects of *Capparis zeylanica* Linn. Leaves; Journal of Ethnopharmacology; 2006; 108(2): 311-315.
3. Bafna AR and Mishra SH, Immunomodulatory activity of methanol extract of roots of *Cissampelos pareira* Linn; Ars Pharmaceutica; 2005; 46(3): 253-262.
4. Ghosh MN, Fundamentals of Experimental Pharmacology; 2nd Edition; Scientific Book Agency; Kolkata; 1984; 156-157.
5. Kulkarni SK; Hand Book of Experimental Pharmacology; 3rdEdition; Vallabh Prakasan; 1999; 117-171.
6. John A Parrotta; Healing Plants of Peninsular India; CABI publishing: 675.
7. https://en.m.wikipedia.org/wiki/Physalis_minima
8. D. Dorcus, Preliminary phytochemical and anti-bacterial studies on *Physalis minima* Linn.; International Journal of Current Sciences; 2012; 24-30 2012,24-30
9. Jyothibasu Tammu *et al*., Diuretic activity of methanolic extract of *Physalis minima* leaves; Der Pharmacia Lettre; 2012; 4 (6):1832-1834
10. MA Khan *et al*., Anti-inflammatory, analgesic and antipyretic activities of *Physalis minima* Linn.; Journal of Enzyme Inhibition and Medicinal Chemistry; 2009; 24(3): 632–637.
11. Patel *et al*.; Study on the antibacterial potential of *Physalis minima* Linn; Indian Journal of Pharmaceutical Sciences; 2011; 111-115.
12. D Daud *et al*., *Physalis minima* Linn methanolic extract reduces blood glucose level without compromising sperm quality in normoglycaemic mice; Journal of Applied Pharmaceutical Science; 2016; 6(6): 008-011.
13. Harborne JB, Methods of extraction and isolation; In: Phytochemical methods; Chapman and Hall, London, 1988; 60-66.
14. Hafeez Bin Bilal, Ahmad Iqbal, Haque Rizwanul, Raisuddin S, Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice; Journal of Ethnopharmacology, 2001; 75(1): 13-18.
15. Wagner H, Plant Drug Analysis. A thin Layer chromatography Atlas, Heidelberg, Springer-Verlag Belgium, 2002; 2nd edition; 227-305.
16. A. Marston, M. Maillard and K. Hostettmann, The role of TLC in the investigations of medicinal plants of Africa, South America and other tropical regions; GIT Laboratory Journal; 1997; 1: 36-39.
17. K Hostettmann, JL Wolfender and S Rodriguez, Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts, Planta Medica; 1997; 63(1): 2-10
18. Makare N, Bodhankar S, Rastogi V, Immunomodulatory activity of alcoholic extract of Magnifera indicaL. in Mice; Journal of Ethnopharmacology; 2001; 78(2-3): 133-137.
19. Joharapurkar AA, Zambad SP, Wanjari MM, Umathe SN, In vivo evaluation of antioxidant activity of alcoholic extract of *Rubia cordifolia* Linn and its influence on ethanol-induced immunosuppression; Indian Journal of Pharmacology; 2003; 35: 232-236.
20. Puri A, Saxena R, Saxena RP, Saxena KC, Immunomodulant agents from *Andrographis Paniculata;* Journal of Natural Products; 1993; 56(7): 995-999.
21. JI Yubin, The extraction, separation and purification of alkaloids in the natural medicine; Journal of Chemical and Pharmaceutical Research, 2014, 6(1): 338-345
22. N Hosoda, Agricultural and Biological Chernistry; 1979; 43(4): 821-825
23. Kurt Hostettmann, Strategy for the Biological and Chemical Evaluation of Plant Extracts Invited lecture presented at the International Conference on Biodiversity and Bioresources: Conservation and Utilization, Phuket, Thailand, November 1997, 23–27

Received:06.08.18, Accepted: 09.09.18, Published:01.10.2018

***Corresponding Author:**
K. Sunitha
Email: kailasasuni@gmail.com