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(RESEARCH ARTICLE)



## Phytochemical, antimicrobial, antioxidant and immunomodulatory studies of leaf extracts of *Cyclea peltata* (Lam.) Hook. f. & Thomson

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### Abstract

The objective of the present investigation is to study the phytochemical constituents, antimicrobial, antioxidant activity and immunomodulatory effects of *Cyclea peltata* leaf extract. The fresh whole plants were collected from Kannur, Kerala, India. The crude extract of leaves of *C. peltata* prepared using methanol and aqueous solvents and were used to investigate the antibacterial activity against *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by well diffusion method. Antioxidant by DPPH method and immunomodulatory study using Swiss albino rats. The extracts showed the presence of phytoconstituents like tannin, proteins, resins, carbohydrates, alkaloids and terpenoids. Both the extracts showed varying degree of inhibitory potential against all the tested bacteria. Methanol extract of plant had higher inhibitory action against *Pseudomonas aeruginosa* and *Streptococcus mutans*. While *Staphylococcus aureus* showed low sensitivity against aqueous extract of *C. peltata*. The body weight of the animals showed constant in the case of lower concentration of the drug (1 mg/kg), whereas at higher concentration, the animals showed reduced body weight, with more reduction in highest concentration. Similarly for the organs like liver, the higher dose showed increased mass of liver when compared to lower doses of the drug. The tested animals showed good immunomodulatory effect against the leaf extract. This study further suggests the use of plant extract in treating disease caused by tested microbial organisms.

**Keywords:** Albino rats; Aqueous extract; *Cyclea peltata*; Immunomodulatory; Methanol extract; Phytochemical constituents

### 1. Introduction

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substance. In the last few decades there is an exponential growth in the field of herbal medicine [1]. It is getting popularized in developing countries owing to its natural origin and lesser side effects. Now-a-days, herbal medicines are being manufactured on a large scale in mechanical units. The manufacturers are facing many problems such as availability of good quality of raw material, authentication, availability of standards, standardization methodology of drugs, formulations and quality control parameters.

Immunology is the study of the immune system and is one of the most rapidly developing areas of medical biotechnology research. The immune system protects us from infection through various lines of defense. It has great promises with regard to the prevention and treatment of a wide range of disorders such as inflammatory diseases of skin, gut, respiratory tract, joints, and central organs. In addition infectious diseases are now primarily considered immunological

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disorders while neoplastic diseases, organ transplantation and several autoimmune diseases may involve immunosuppressive state.

Immunomodulators are natural or synthetic substances that help to regulate or normalize the immune system. Immunomodulators correct immune systems that are out of balance. Natural immune modulators are less potent than prescription immune modulators and also less likely to cause side effects. Prescription immunomodulator medications, such as azathioprine, 6-mercaptopurine and methotrexate work by suppressing the immune system and decreasing inflammation in the digestive tract in people with bowel disease, ulcerative colitis and Crohn's disease. The benefits of immunomodulator system from their ability to stimulate natural or adaptive defence mechanisms, such as cytokines, which enables to help the body itself.

Lymphocytes are one of many types of white blood cells produced in the bone marrow by the process of hematopoiesis. Lymphocytes leave the bone marrow, circulate in the blood and lymphatic system, and reside in various lymphoid organs. Because they produce and display antigen binding cell surface receptors, lymphocytes have the defining immunologic attributes of specificity, diversity, memory, and self/nonself recognition. The two major populations of lymphocytes are B lymphocytes and T lymphocytes [2]. Phansalkar et al. [3] studied immunomodulatory effect of *Withania somnifera* (L.) Dunal in mice with myelosuppression induced by one or more of the three compounds like cyclophosphamide, azathioprin or prednisolone by hematological and serological test. Li et al. [4,5] studied the immunomodulatory effect of polysaccharides and showed that the polysaccharide may prime and trigger M phi and has restorative effects on the deficiency of the immune system associated with aging in mice, rats and piglets. Various investigators studied the immunomodulatory effect of different solvent extracts of plant species in mice [6-13]. The objectives of present investigation is to analyse the phytochemical constituents, to evaluate the antimicrobial and antioxidant activity and to study the immunomodulatory activity of aqueous extracts of *Cyclea peltata* leaves on Albino rats.

## 2. Material and methods

### 2.1. Plant material

*Cyclea peltata* (Lam.) Hook. f. & Thomson belongs to family Menispermaceae, is a much-branched, climbing shrub (Plate1A) found throughout South and east India and in the Andaman and Nicobar Islands. Roots are tuberous; Leaves are peltate, hairy, 2- 5 cm long, flowers are very small. Male flowers in panicles are very long. Fruits are drupe and reniform (Plate1B). The species of this plant are easily distinguished by the cup-shaped calyx and corolla. The plant contain the chemical constituents like fangchinoline, cycleapeltine, cycleadrine, cycleaurine, cycleanorine, cycleahominechloride, chondocurine, mangoflorine, Isotetradrine, perpamine, cycleamine and burmannaline. The plant is widely used in the treatment of cough, fever, kidney disorder, urinary disorder and snake poisoning. Powdered roots for the treatment of diabetes, tooth ache; decoction of the roots and leaves used for treating malaria and asthma.



**Figure 1A** Habit of *Cyclea peltata*, **1B** Fruits of *C. peltata*

The plant leaves were collected from Kannur, Kerala in the month of September 2018. The plant was confirmed in the department; the specimen's herbarium was prepared with number ALCM/PGBT 10 and deposited in the department of PG Biotechnology.

The collected leaves were cleaned with tap water and shade dried for a week. By using the kitchen blender the plant material was grounded to a fine powder form. The powder was then processed for extraction using solvents.

## 2.2. Preparation of extract of *C. peltata*

Eighty grams of powdered sample was mixed with 800 ml of distilled water and boiled for 45 minutes. It was then filtered and the filtrate was kept in water bath for evaporation. The concentrated extract was then transferred to pre-weighed china dish. The extract was allowed to dry. This was repeated three times. Ten grams of the powder was taken in 100ml of methanol for extraction. It was then filtered and stored at 4°C.

## 2.3. Phytochemical screening of plant extract

Qualitative tests were carried out for the preliminary screening of phytochemicals like carbohydrates, alkaloids, tannins, resins and saponins in the aqueous, methanol extracts reconstituted in respective solvents using standard procedures to identify the constituents [14].

## 2.4. Antibacterial screening of the leaf extracts

### 2.4.1. Preparation of inoculums

A loop full of culture was inoculated into nutrient broth and incubated at 37°C for 24 hours to obtain a bacterial culture. This procedure was carried for the selected bacterial cultures to obtain inoculums of particular broth culture. Bacterial cultures were collected from the P.G. department of Biotechnology. The strains are *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

### 2.4.2. Antimicrobial activity by agar well diffusion method

Petri dishes were plated with Nutrient agar media and allowed to solidify for 30 minutes. The test organisms were then spread on the surface of the media using sterile ear buds. Cork borer (4mm) was used to bore wells in media. 50µl of aqueous extract of *Cyclea peltata* with different concentrations i.e., 500, 800, 900, 1000µg/ml was dispensed into the well using a micropipette respectively. A negative control of distilled water and a positive control of 20µg/ml Penicillin G were kept and the extract was allowed to diffuse for half an hour at room temperature. Then the plates were incubated at room temperature for 24 hours. The zones of inhibitions were measured. For each organism three replicates were made.

## 2.5. Antioxidant activity by DPPH method [15]

Aqueous extracts of *Cyclea peltata* leaves were prepared. Two concentrations (300µg/ml and 600µg/ml) were prepared by using water. 2.5 ml of sample was transferred into a test tube. To that 2.5 ml of DPPH was added and mixed well. It was then incubated at room temperature in darkness for 30 minutes. Absorbance was then measured at 520nm using a mixture of distilled water and absolute ethanol as blank.

$$\% \text{ inhibition} = \frac{\text{Control absorbance (Ac)} - \text{Sample absorbance (As)}}{\text{Control absorbance (Ac)}}$$

## 2.6. Immunomodulatory activity

### 2.6.1. Animals

Random bred Swiss albino rats (100-150g body weight) of both sexes were used for immunological studies. Animals were randomly divided into 4 treatment groups (minimum 5 animals per group) based on the concentration of the drug administered. Animals were maintained in cages with paddy husk as bedding. Animals were housed at temperature 24 ± 2°C, 12 hour light or dark cycle and fed with standard pellet diet and water ad libitum. The experiments were started after obtaining the Institutional Ethical committee consent.

Group	No. of animals
Group 1(Control)	5
Group 2(1mg/kg)	5
Group 3(2mg/kg)	5
Group 4(4mg/kg)	5

### 2.6.2. Dosage

The animals were divided into four groups. Each group comprised of a minimum of 5 animals. The plant extract was suspended in water and was administered orally for 14 days. Group I (control) received water; group II plant extract @ 1 mg/kg body weight; group III, @ 2 mg/kg; and group IV received 4 mg/kg weight of the animal. For animal experiment methods of Bin-Hafeez et al. [16] was followed. The dose volume was calculated to be not more than 1 ml of drug preparation per animal. Control animals received 1 ml of water.

### 2.6.3. Immunization

Sheep RBC (SRBC) were collected in Alsever's solution, washed three times in phosphate buffered saline (PBS) and adjusted to a concentration of  $0.5 \times 10^9$  cells/ml. On the 7th day all the rats were immunized with  $0.5 \times 10^9$  cells/ml of SRBC peritonally using an insulin syringe. This is considered as the first dose. On the 11th day a booster dose was given.

### 2.6.4. Body weight and lymphoid organ weight

The animals were humanized after 14th day of treatment. Before sacrificing the animals, body weight of all the animals were recorded and blood was collected by heart puncturing using 21 gauge needle and syringe. The collected blood was used for performing different tests such as total haemocyte count, differential count, blood glucose and hemoglobin. The serum was also prepared to run an assay for hemagglutinating antibody titre and serum albumin and globulin. After collecting blood, animals were sacrificed and the weight of organs like liver, thymus and spleen were recorded. Lymphoid tissues like, spleen, thymus including liver and kidney were fixed in phosphate buffered formalin for histological investigations to be conducted as a continued part of this work in a later period.

### 2.6.5. Blood glucose test (Trinder's method)

Three test tubes were taken and marked as B (blank), T (test), and S (standard). 1 ml of glucose solution was added to all the tubes. To the tube 'T' 0.01 ml of sample plasma was added. 0.01 ml of standard glucose was added to the tube S. All the tubes were incubated at 37°C for 5 minutes. Absorbance was read at 630 nm in a UV-Vis spectrophotometer (Systronics, 2202).

### 2.6.6. Hemoglobin test (Cyanmethemoglobin method-CMG method)

As much as 5 ml of Drabkin's reagent was added to the tubes marked as B (blank), S (Standard) and T (Test). Exactly 0.02 ml of blood was added to the tube T. CMG standard up to a volume of 0.02 ml was added to the tube S. Then 0.02 ml of distilled water was added to the tube B. All the tubes were incubated at room temperature for 10 minutes. The absorbance was read at 540 nm in UV-Vis spectrophotometer.

### 2.6.7. Determination of total serum protein and albumin: globulin ratio

This was estimated by Biuret method and absorbance was read at 540 nm in a UV spectrophotometer.

### 2.6.8. Total leukocyte count and Differential count of white blood cells

Total count was determined by WBC diluting fluid using Hemocytometer. Blood smear was prepared on a clean glass slide, allowed to dry and was fixed in methanol for 3 minutes. The slide was dried and dipped in Field's solution B for 5 seconds. It was then rinsed with water and allowed to dry. Then it was stained with Field's solution A for 15 seconds. After staining slide was rinsed with water and dried again. Stained slide was then observed under microscope.

### 2.6.9. Hemagglutination antibody titer [17]

As much as 100  $\mu$ l of serum was heat inactivated at 56°C in water bath for 30 minutes. About 50  $\mu$ l of PBS was added to all 12 wells of microtiter plate row. First well was taken as control and was not added with serum, instead it received only PBS. Next well received 50  $\mu$ l of heat inactivated serum. From the same well using a micropipette, 50  $\mu$ l of the mixture was taken after completely mixing it with the pipette and is serially diluted by 2 fold in the subsequent wells. Finally 50  $\mu$ l of SRBC with a cell density of  $0.5 \times 10^9$ /ml was added to all the wells. The plate was gently tapped to mix the cells and was incubated at 37°C for 2 hours. The value of antibody titer was assigned to the highest serum dilution showing at least 50% of visible hemagglutination.

### 2.6.10. Hypersensitivity reaction

On the day of termination of the treatment, animals were sensitized with  $0.025 \times 10^9$  cells/ml of SRBC on the right hind footpad by gently injecting the same using a insulin syringe. Increase in footpad thickness of rat was measured after 3 hours of the treatment and increase in volume of foot pad was measured manually.

## 3. Results

The phytochemical screening of extracts of *Cyclea peltata* was carried out by following the standard procedures. Aqueous extract showed the presence of carbohydrates, alkaloids, saponins, tannins, resins and terpenoids, where as the methanol extract showed the presence of all the above mentioned phytochemicals except carbohydrates (Table 1).

**Table 1** Phytochemical analysis of extracts of *Cyclea peltata* leaves

	Aqueous extract	Methanol extract
Carbohydrates	+	-
Tannins	+	+
Alkaloids	+	+
Terpenoids	+	+
Resins	+	+
Saponins	+	+

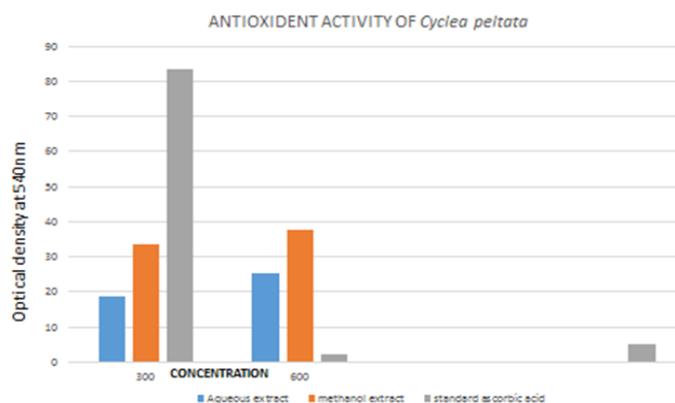
'+' indicates present, '-' indicates absent

The bacterial cultures showed various levels of sensitivity towards different concentration of aqueous extracts of *Cyclea peltata*. *Pseudomonas aeruginosa* and *Streptococcus mutans* showed high sensitivity against the aqueous extract of *C. peltata* (3.9 cm). *Staphylococcus aureus* showed low sensitivity against aqueous extract of *C. peltata* (3.6 cm) (Table 2).

**Table 2** Antibacterial activity of the aqueous extract of *Cyclea peltata* at different concentration

Bacterial strain	Diameter of zone of inhibition (cm)					
	500µg/ml	800µg/ml	900µg/ml	1000µg/ml	Positive	Negative
<i>Klebsiella pneumoniae</i>	1.6	3.2	3.5	3.7	1.9	-
<i>Staphylococcus aureus</i> ATCC29213	2.3	3.5	3.6	3.6	1.8	-
<i>Streptococcus mutans</i> MTCC 497	2.6	3.4	3.7	3.9	1.5	-
<i>Escherichia coli</i> ATCC25922	1.8	3.4	3.6	3.8	-	-
<i>Lactobacillus acidophilus</i> MTCC10307	0.9	1.8	2.9	3.8	-	-
<i>Pseudomonas aeruginosa</i>	1.9	3.9	3.9	3.9	1.4	-

The aqueous extract of *Cyclea peltata* exhibited lower antioxidant activity compared to the standard ascorbic acid. The aqueous extract of leaves of *C. peltata* showed 18.8% inhibition at 300mg/ml and it showed 33.5% inhibition in 600mg/ml, while the methanolic extracts showed 33.5% inhibition at 300mg/ml and 37.6% inhibition in 600mg/ml compared to 83.3 and 88.6% of standard ascorbic acid (Fig. 2).



**Figure 2** Antioxidant activity of *Cyclea peltata* leaf extract

During the study various morphometric, haematological, serological and other parameters required to assess the immune status of the albino rats upon administration of *Cyclea peltata* drug formulation for 14 days were recorded. The body weight of the animals of control group and that of treatment groups are given in the table 4. The table also shows the weight of different vital lymphoid organs taken at the time of humanization after 14 days of treatment. The body weight of the animals showed constant in the case of lower concentration of the drug (1 mg/kg). However, at higher concentration, the animals showed reduced body weight, with more reduction in highest concentration. Similarly for the vital organs like liver, the higher dose showed increased mass of liver when compared to lower doses of the drug.

**Table 4** Morphometric measures of albino rats treated with extracts of *Cyclea peltata*

	Body weight (g)		Liver (g)	Spleen(g)	Kidney(g)
	Initial	Final			
Group 1	200	200	07.2	0.86	0.81
Group 2	215	215	08.06	0.90	0.97
Group 3	200	200	09.2	01.0	01.02
Group 4	200	170	09.5	01.2	01.17

The total leukocyte count and differential count are showed in table 5. When compared to control group total leukocyte count was found increased in the lowest concentration (1 mg/kg) of drug administration. However increased in the drug concentration has further decreased the total leukocyte count. The percentage of neutrophils was found highest in lowest concentration (1 mg/kg) of drug tested with lowest percentage in highest concentration of a drug.

**Table 5** Hematological parameters of albino rats treated with extracts of *Cyclea peltata*

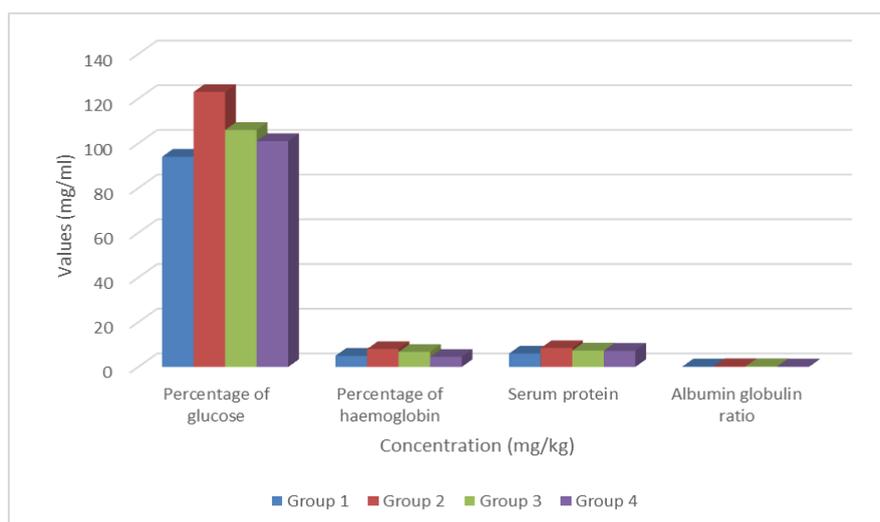
Groups	Leukocyte count	Total Differential Count				
		Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil
Group 1	2050	40	4	52	3	1
Group 2	2750	33	6	56	3	1
Group 3	2450	44	6	46	2	1
Group 4	2300	40	6	50	3	1

The glucose level is found to be at higher concentration of 123 mg/dl in lowest concentration group (1 mg/kg). Whereas increased concentration of the drug had reduced the blood glucose level. Similar trend is noticed for hemoglobin, serum protein and albumin globulin ratio. Hemoglobin is found to be highest in group I where drug administration was 1mg/kg (Table 6, Fig. 3).

**Table 6** Hemoglobin and serum protein

Groups	Percentage of glucose(mg/dl)	Percentage of Hemoglobin(g/dl)	Serum protein (g/100ml)	Albumin globulin ratio
Group 1	94	5.0	6.0	6:1
Group 2	123	8.0	8.4	8:1
Group 3	106	6.7	7.3	7:2
Group 4	101	4.5	7.2	7:1

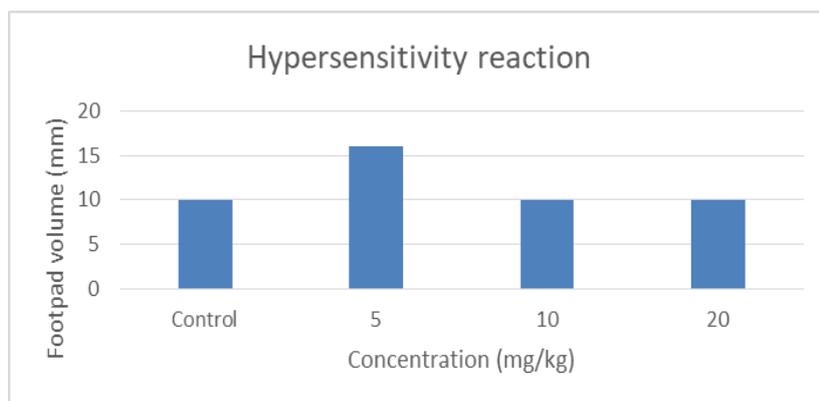
The results of HA titer is given in the table 6. After the immunization of the animals both for the control and treatment groups, hemagglutination antibody titer were estimated. Among the animals of group I and II which received drug at concentration 1 mg/kg and 2 mg/kg respectively showed highest antibody titer against SRBC. However, the increased concentration of drug administered did not show proportionate increase in the hemagglutination antibody titer.

**Figure 3** Serum protein levels

Delayed hypersensitivity reaction was noted highest in group I which received the lowest concentration (1 mg/kg) of drug when compared to control group. In this study a remarkable increase in paw volume was seen in case of drug administered at the rate of 4 mg/kg/day. In case of other groups hypersensitivity reaction could not be distinguished from that of the control group (Table 7 and Fig. 4).

**Table 7** Haemagglutination antibody titer and hypersensitivity reaction

Groups	Haemagglutination antibody titer	Hypersensitivity reaction (Percentage increase in paw volume)
Group 1	1: 3096	10
Group 2	1: 5536	16
Group 3	1: 5536	10
Group 4	1 :1034	10



**Figure 4** Hypersensitivity reaction

#### 4. Discussion

Preliminary phytochemical screening for the methanol and aqueous *Cyclea peltata* leaf extract was carried out by following the standard procedures. Aqueous extract showed the presence of carbohydrates, alkaloids, saponins, tannins, resins and terpenoids whereas methanol extract showed the presence of all the above mentioned phytochemicals except carbohydrates and tannins. In one of the earlier experiment Hullatti et al. [18] screened petroleum ether and ethanolic extracts of *C. peltata* for phytochemicals and revealed the presence of phytosterols and alkaloids as major phytoconstituents in petroleum ether extract. The ethanolic extract showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins. Shine et al.[19] reported the presence of alkaloid content in all extracts, whereas saponins, steroids and terpenoids were detected in CP and CCP of *C. peltata*. Similar results were reported in other medicinal plants earlier [20, 21].

The antibacterial activity of the tested extracts of *C. peltata* showed significant reduction in bacterial growth in terms of zone of inhibition. The observed antimicrobial activity against the test organisms could be due to the presence of phytochemicals in the extract. The bacterial cultures showed various levels of sensitivity towards different concentration of aqueous extracts of *C. peltata*. *Pseudomonas aeruginosa* and *Streptococcus mutans* showed high sensitivity against the aqueous extract of *C. peltata*. *Staphylococcus aureus* showed low sensitivity against aqueous extract. Petroleum ether, hexane, chloroform, ethyl acetate, acetone, methanol and aqueous extracts of five concentrations (1, 2, 5, 10 mg/ml) were used to investigate the antibacterial activity. All the extracts showed varying degree of inhibitory action against *Staphylococcus haemolyticus*, *Klebsiella pneumoniae* and *Proteus vulgaris*. Acetone extract showed maximum inhibitory action against *Klebsiella pneumoniae* and *Streptococcus haemolyticus* [22].

*Cycleapeltata* which has repeatedly been termed as a medicinal plant in the folk medicine is used in this study to analyse its antioxidant potential. The antioxidant activity of the plant was studied by the DPPH method. The stable nitrogen centered free radical. The aqueous extract of *Cyclea peltata* is showed antioxidant activity which was compared to the standard ascorbic acid. In the present study, the aqueous leaf extract of *C. peltata* showed 18.8% inhibition at 300mg/ml and it showed 33.5% inhibition in 600mg/ml, while the methanolic extracts showed 33.5% inhibition at 300mg/ml and 37.6% inhibition in 600mg/ml. Vijeesh et al. [21] found that the extracts even possesses antifungal activity too.

Chellappan et al. [23] evaluated the extracts of leaves and roots of *C. peltata* for physico-chemical, preliminary phytochemical analyses and antioxidant properties. Acute oral toxicity study was conducted as per OECD-423 guidelines and the extract was found to be devoid of any conspicuous acute toxicity in extract treated animals and no mortality up to 2 g/kg by oral route. Hydroalcoholic extract of *C. peltata* in a dose dependant manner (125 and 250 mg/kg. p.o.) showed significant gastric protection against the ethanol-induced gastric ulcer model in rats. Different extracts of *C. peltata* were used for the experiments including anti-inflammatory by Shine et al. [19]. ACP and TET showed significant *in vitro* anti-inflammatory and antioxidant activity when compared to other extracts. ACP and TET (100 µg/ml) treatment significantly inhibited the mRNA expression of iNOS, COX-2, TNF-α in LPS treated RAW 264.7 cells. HPTLC estimation of bioactive compound tetrandrine was highest in ACP-228.4 µg/mg followed by CP-29.62 µg/mg, CCP-23.46 µg/mg, MCP-18.82 µg/mg and HCP-1.25 µg/mg. TET has been isolated from ACP.

The antioxidant activity of methanolic extracts of various parts of *Lantana camara* showed that all the plant parts possessed antioxidant properties including radical scavenging, xanthine oxidase inhibition and nitrites scavenging

activities were correlated with the total phenol. The leaves extract of *L. camara* was more effective than that of other parts as well as in other plants too [24-27].

*Cyclea peltata* aqueous extract when administered to the albino rats at varying concentration have shown effect on their body weight and relative weight of vital organs like liver. The body weight remains constant (during 14 days trial) among the group 1 which received 1 mg/kg of test drug. However, with increase in concentration of the drug there was gradual reduction in the body weight indicating that drug is growth retarding instead of growth enhancing at concentration above 2 mg/kg body weight. The results are also strengthened with data obtained from liver which is a vital organ that respond immediately to any drug or toxin. In the present study the highest concentration (32 mg/kg) showed the highest liver mass showing possible hyperactivity at liver to ameliorate the toxic effect of the drug administered. Incidentally the lower concentration (1 mg/kg) of the drug showed the relative mass of liver to be similarly to control showing normal activity of liver. In case of thymus no difference among the control and treatment group were noted. Bin-Hafeez et al. [16] studied immunomodulatory effect of fenugreek extract in mice showed that no significant in body weight gained could not be noted among the animals there were no effect on spleen. Similar conclusions made by them which is comparable to present study. They found increased mass of liver in concentration up to 100 mg/kg and however they have opined that increase in liver mass could not be correlated to any other toxic effect as revealed by assays on some enzymes. One of the earliest immune response can be seen and measured by studying the hematological parameters of an animal. Accordingly parameters like total leukocyte count and differential count were measured for control group as well as group which received various concentrations of drug. Blood cells are the first cells to be responds to invading non-self-materials.

In the present study group 1 which received lower concentration (1 mg/kg) of drug showed highest leukocyte count of 2750 WBC/mm<sup>3</sup> showing the initial triggering of blood cell to mount a potent immune response. The results showing lowered concentration of drug (1mg/kg) are better to elicit good immune response than higher concentrations (2mg/kg, 4mg/kg) of drug administered. The results are further strengthened with highest percentage of neutrophil being circulated in the group receiving lower concentration (1 mg/kg) of drug.

Serum protein and serum albumin globulin ratio is one of the earliest indicators of normal serum chemistry of an individual. A change in serum protein concentration and albumin ratio would hint us about the altered immune response status of the individual. Accordingly in the present study serum protein level and albumin globulin ratio is found to be similar in case of control and higher concentration (4 mg/kg) of the drug but in the lower concentration (1 mg/kg) of drug test the group showed increase in serum protein and albumin ratio showing that higher immune response might have contributed to the serum protein in terms of different molecules such as immunoglobulins and other humoral factors. Similar results were observed for glucose and hemoglobin. Hemoglobin is also one of the important parameter that would reveal the health status of the individual. So in the present case group 1 receiving the lowest concentration (1 mg/kg) of the drug show a better health index based on hemoglobin. In one of the earlier studies on leaf extracts of *Moringa oleifera* on Wistar rats by Nfambi et al. [28] revealed an increment in WBC, lymphocyte, and neutrophil counts at a dose of 1000 mg/kg body weight similar to the levamisole-positive control group. The neutrophil adhesion was statistically significant ( $p \leq 0.05$ ) for treatment groups that received 1000 mg/kg body weight (29.94%) and 500 mg/kg body weight at 17.28%. The mean percentage increment in footpad thickness was highest (26.9%) after 8 h of injection of antigen in the footpad of rats dosed 500 mg/kg body weight and this later reduced to 25.6% after 24 h. There was a dose-dependent increment in the mean hemagglutination antibody titer to sheep red blood cells (SRBC) from  $10.73 \pm 0.57$  HA units/ $\mu$ L for the 250 mg/kg body weight to  $26.22 \pm 1.70$  HA units/ $\mu$ L for the 1000 mg/kg body weight.

Hemagglutination antibody titer assay is one of the key parameter used to assess the humoral immune response of the animal. As the antigen is expected to induce the production of antiserum against it, in the present study sheep red blood cells were used to elucidate the production of antibody against RBC. In an individual where immune system is primed antibody against a particular antigen is expected to be at higher titer. Accordingly in the present study a very high hemagglutination antibody titer was recorded and group 2 and group 3 individual which received the lowest concentration (1 mg/kg and 2 mg/kg) of test drug. On the contrary higher concentrations of the drug have surprisingly reduced the HA titer. Pradhan et al.[29] administered extract of herbal product to albino rats showed a increased HA titer when drug was used at a concentration of 50mg/kg. Similarly Bin- Hafeezet al.[16] also showed increased HA tire at doses of 50 mg/kg and above, of fenugreek extract administered on mice. They recorded the HA titer up to 1:2389. Further Fulzele et al.[6] studying immunostimulant activity of *Ashtamangala ghrita* in rats showed that HA titer could be well employed to study humoral immunomodulatory activity among the animals treated with drug. The authors showed that up to 300mg/kg of the crude drug could enhance the humoral immune response. Upon examining the present results it is evident that the aqueous extract of *Cycleapeltata* at concentrations less than 2 mg/kg induced humoral immune response as evidenced by HA titer. Prasad et al.[30] used delayed type hypersensitivity assay to

evaluate immunomodulatory activity of *Momordica charantia* ghrita extract on albino rats. There was an increase in paw volume in case of drug administered at the rate of 350 mg/kg/day. Pradhan *et al.* [28] also showed an increased hypersensitivity reaction in case of rats administered with herbal drugs. In the same line of earlier results, Bin-Hafeez *et al.* [16] also showed a significant increase in delayed type hypersensitivity at a concentration at 50 mg/kg compared to control. On the other hand Fulzele *et al.* [6] showed *Ashtamangala ghrita* could also elucidate increased delayed type hypersensitivity response. Venkatachalam *et al.* [31] reported delayed hypersensitivity at 400mg/kg dose of *Morus alba* aqueous extract on Wistar rats and thereby induced a better immunomodulatory activity.

In one of the study by Bafna and Mishra [10] the alkaloidal fraction of roots of *Cissampelos pareira* showed significant immunosuppressive activity at lower doses (25 and 50 mg/kg) while no activity was observed at higher doses (75 and 100 mg/kg). Humoral antibody titre was significantly ( $p < 0.01$ ) lowered by AFCP at the doses of 25 and 50 mg/kg. Delayed type hypersensitivity response was also significantly ( $p < 0.01$ ) suppressed by the AFCP at the dose of 75 mg/kg. Hence they concluded the immunosuppressive and antioxidant activities of the alkaloidal fraction of *C. pareira* roots. Immunomodulatory activity of methanolic extract of *Murraya koenigii* (L.) Spreng. leaves increased the antibody titre against the ovalbumin and protection towards the cyclophosphamide induced myelosuppression. However, the extract did not show any significant increase in delayed type hypersensitivity reaction which indicated the inability of the extract to stimulate T cells [32]. Present study thus reveals that the extract holds promise as immunomodulatory agent, which acts by stimulating humoral immunity and phagocytic function. Similarly, Nfambi *et al.* [28] found a significant immunostimulatory effect on both the cell-mediated and humoral immune systems in the Wistar albino rats treated with methanolic leaf extract of *M. oleifera*. There are other earlier reports supports the present investigation [1, 19, 23, 33, 34].

The overall results of the present study showed that the immune response is clearly boosted upon on administration of aqueous extract of *Cyclea peltata*. It is evident from the results that lower concentration of drug extract particularly 1mg/kg body weight has the potential to trigger the both humoral and cellular immune response. Contrarily 2mg/kg body weight or more has yielded a negative response in terms of mounting an immune response. Though these kinds of results have not been encountered in the literature it is not uncommon to have a varied physiological function of a plant derivative. There were few earlier works on this species adds support to the present study as it enhances immunomodulatory effect [19, 35, 36]. A drug, as per the science of herbal drug can result in different action based on the concentration of the drug prescribed. It is very common in Ayurvedic practices were concentration dependent effect is achieved as per the requirement. Therefore, there is every possibility that a aqueous extract of *Cyclea peltata* might trigger a toxicological or a negative effect on any of the physiological or immunological system of the body. Nevertheless the drug tested has evidently shown the immunomodulatory effect when used at lower concentration. As a concluding mark *Cyclea peltata* could be employed to boost immune system at low concentration and it should also be kept in mind that high concentration of same could lead to physiological effect in the body.

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## 5. Conclusion

*Cyclea peltata* possesses antimicrobial and antioxidant activity due to the presence of phytochemicals in it. Therefore, there is every possibility that an aqueous extract of *C. peltata* might trigger a toxicological or a negative effect on any of the physiological or immunological system of the body. As a concluder mark *C. peltata* could be employed to boost immune system at low concentration and it should also be kept in mind that high concentration of same could lead to physiological effect in the body. Literature on this plant on any other health benefit effect or immunomodulatory activities is not available. Therefore the present findings on the immunomodulatory studies deduced that, the albino rats administered with varying concentrations of aqueous extracts of *C. peltata* would give a base line data and scientific evidence of immunological properties of the chosen plant.

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## Compliance with ethical standards

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### Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

*Statement of ethical approval*

The animal based experimental study was approved by the Institutional Ethical committee.

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