

Phytochemical Screening, Spectroscopic Characterisation and Cardiostimulatory Activity of Aqueous Extract from Aerial Parts of *Carmona Retusa* (Vahl) Masam on Isolated Frogs Heart

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Abstract

The present study was undertaken to evaluate cardiostimulatory activity of aqueous extract of whole plant of *carmona retusa* (Vahl) masam. Phytochemical screening of whole plant material revealed that the major constituent of Carmona retusa (Vahl.) Masam plant is an intractable mixture of alkaloids, triterpenes, flavanoids and cardiac glycosides. Cardiostimulatory effect of aqueous extract of whole plant of *carmona retusa* was studied by using isolated frog heart perfusion technique (IFHP). Calcium free Ringer solution was used as vehicle for administration of aqueous extract of *carmona retusa* as a test extract and digoxin as a standard. A significant increase in height of force of contraction (positive inotropic effect) and decrease in heart rate (negative chronotropic effect) at a very low concentration (10µg/ml) was observed with test extract as compared to the same dose of a standard digoxin. The present results indicated that a significant increase in height of force of contraction with decrease in heart rate was observed as the dose of test extract increased. The test extract does not produced cardiac arrest at 80µg/ml, a higher concentration, as compared to standard, digoxin, a drug with narrow therapeutic window, *carmona retusa* showed wide therapeutic window.

Keywords: *Carmona retusa*, aqueous extract, phytochemistry, cardiostimulatory activity

1. Introduction

Cardiovascular diseases have been considered a severe health problem around the globe. The major risk factors for heart diseases include family history, sex, hypercholesterolemia, hypertension, obesity, and cigarette smoking. Most of these risk factors are prevalent in developing countries because of the absence of appropriate infrastructure. Therefore, these diseases have become a very common problem in the rich population of the developing countries [1].

India is enriched with large variety of medicinal plants. According to a survey report more than 5000 species of plants with probable potential of pharmacological activities are distributed throughout Himalayan range in India. Out of these 5000 species, about 600 to 700 species are being used by local people for many medicinal purposes [2]. It is understood that synthetic medicines cause more side effects as compared to natural products [3]. Finding healing powers in plants is an ancient idea, in this respect herbs have been used for medical treatment since the beginning of human civilization [4]. The Boraginaceae is a family with a large number of plants. It has 50 genera and 2000 species which are widely distributed throughout the northern hemisphere. It also found in southern temperature regions [5]. One of Boraginaceae important member is *carmona retusa* traditionally used as a disinfectant wash during childbirth, leaves has been used as cure for diarrhea, as tea for general good health and because Tsang Gubat has high fluoride content, it is used as a mouth gargle for preventing tooth decay [6].

Carmona retusa (Vahl) Masam [7,8] Fam.-Boraginaceae (Heliotrope family) previously known as *Ehretia microphylla* Lam. Carmona is a monotypic genus [9]. The only species of *Carmona retusa* is found from India eastward to southern China, Taiwan and Japan, and further south throughout Malaysia to New Guinea and the Solomon islands. Synonyms: *Ehretia microphylla* Lam., *Ehretia buxifolia* Roxb., *Carmona microphylla* (Lamk) G.

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Don., *Cordia retusa* Vahl, *Carmona retusa* is a beautiful shrub (small tree) with many erect branches covered with glossy dark green coarse leaves. *Carmona retusa* (Vahl) Masam (*Ehretia microphylla* Lam.) is reported to be medicinally useful in Indigenous System of Medicine [10]. A novel natural product microphyllone has been isolated from *Ehretia microphylla* together with baurenol and ursolic acid[9]. The effects of *E. microphylla* promote the pituitary-ovary axis activities and cause an elevation in the serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol hormones, as well as increase the mean numbers of follicles and eventually ovarian weight [11]. The leaves are used as a stomachic, and in the ailments of cough, fever and constitutional syphilis. The roots are used in southern India for Cachexia and syphilis and as an antidote for certain vegetable poisons [12,13]. *In-vitro* anti-inflammatory activity of alcohol extract of stem of *Carmona retusa* was investigated by human red blood cell membrane stabilization method and shows it as a potential source of anti-inflammatory agents [14,15]. *C. retusa* has a high potential infighting the growth and multiplication of cancer cells [16,17]. However there is no scientific data on the use of this plant on Cardiotonic activity. Hence the present study was carried out to evaluate the effect of aqueous extract of various arial parts of *C. retusa* on isolated frog's heart. The activity of the aqueous extract was found to be effective.



Figure 1: *Carmona retusa* plant

2. Materials and Methods

2.1 Collection of plant material

The whole plant *C. retusa* was collected from Lxmipuram, Guntur District, Andhra Pradesh, India. The plant was identified by Prof. Dr. Sandhya Rani, Department of Botany SIMS College of Life Sciences, Guntur District.

2.2 Preparation of the plant extract

The various parts of the plant such as root, stem and leaves were shade dried and made into coarse powder. 500g of coarse powder of root, stem and leaves were extracted with distilled water and filtered using whatmann No. 1 filter paper. The filtrates were concentrated on water bath and finally in vacuum. The yield of aqueous extract was found to be 60%. The thick dark brown / dark green paste of aqueous extract of *C. retusa* was stored in air tight container at 4°C till further use. These extracts were used for the evaluation of cardiotonic activity.

2.3 Preliminary evaluation tests

2.3.1 Phytochemical Screening

The aqueous extract of *carmona retusa* plant was subjected to preliminary phytochemical tests and analysed for the presence of various bioactive chemical constituents such as glycosides, alkaloids, steroids, proteins and triterpenoids, carbohydrates, flavonoids[18]. Results were reported in Table-1.

1. Test for steroids

Salkowski test: Few drops of H_2SO_4 is added to the plant extract, shaken the lower layer turns red in color it indicates the presence of steroids.

2. Test for triterpenoids

Libermann buchards test: To the chloroform solution of extract, few drops of acetic anhydride added from the sides of test tube a reddish brown ring is observed at the junction of two layers indicates the presence of triterpenoids.

3. Test for saponins

Foam test: Small amount of extract is shaken with little quantity of water, and then foam was produced, persists for 10min it confirms the presence of saponins.

4. Test for alkaloids

Wagners test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate indicates the presence of alkaloids.

5. Test for carbohydrates

Benedicts test: The extract on heating with Benedict's reagent, brown precipitate was observed indicating the presence of sugar.

6. Test for cardiac glycosides

Keller-killiani test for cardiac glycosides: Chloroform extract of plant and glacial acetic acid with ferrous chloride and 0.5ml of Conc. H₂SO₄ the acetic acid layer shows blue color indicating the presence of glycosides.

7. Tests for phenolic compound

Ferric chloride test: Treated the extract with ferric chloride solution and blue color appeared indicating the presence of hydrolysable tannins.

2.3.2 Fluorescence analysis

Fluorescence analysis was carried out as per the standard procedure. In the present study powdered aerial parts of plant (leaf, stem, fruit) was treated with ethyl acetate, distilled water, 1N NaOH, 1N HCl, 50% H₂SO₄. These extract was subjected to fluorescence analysis in visible/day light and UV light (465nm & 254nm) and reported Table-2.

2.3.3 Thin Layer Chromatography

The TLC was performed on precoated 20×20 cm and 0.25 mm thick plates. The plates were Prepared by using silica gel G for TLC, were left overnight for air drying. These plates were activated by hot air oven at 100°C for 2hr; aqueous extract was plotted on TLC plates [19]. The Plates were dried and developed in 10ml of suitable solvents for rapid screening chloroform / methanol in the ratio 6:4. The plates were run in the above solvent systems and dried at room temperature. Derivatization of TLC plates was done by UV light at 254nm. Different bands were observed and corresponding R_f values are determined. R_f value of each spot was calculated as:- $R_f = \text{Distance travelled by the solute} / \text{Distance travelled by the solvent}$.

2.3.4 UV-Visible Spectral Analysis

The extracts were examined under UV and Visible light for proximate analysis. The sample is diluted to 1:10 with the same solvent to detect the UV-Visible spectrum profile of crude extracts of *carmona retusa* the extracts were scanned in the wave length ranging from 200- 1100 nm by using spectrophotometer and the characteristic peaks were detected.

2.3.5 FTIR Studies

The FTIR Spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. The aqueous extract of *carmona retusa* (vahl) masam was subjected to IR analysis the results confirmed the presence of various types of functional groups.

3. Evaluation of Cardiotonic activity

3.1 Preparation of infusion

1gm of powdered extract was mixed with 100ml distilled water with the help of magnetic stirrer for half an hour. The material was filtered through Whatman filter paper no.40 and filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportion as follows 10, 20, 40 µg/ml. All the preparations were evaluated for their cardiotonic activity by using isolated frog heart assembly. The rate and force of heart contraction was determined.

3.2 Preparation of digoxin solution

The marketed digoxin ampoules (Sunpharma Ltd.) were obtained from local market. Various dilutions were made with distilled water as follows 10, 20, 40 µg/ml. Standard dilutions were evaluated for their cardiotonic activity and treated and recorded on a Sherrington Kymograph.

3.3 Preparation of hypodynamic ringer solution

Hypodynamic ringer solution was prepared by using standard method [20].

3.4 Cardiotonic activity

The frog of species *Rana tigrina* selected to evaluate cardiotonic activity [21] was pithed and pinned it to the frog board. A midline incision was given on the abdomen, the pectoral girdle was removed and the heart was

exposed. The pericardium was carefully removed and put a few drops of hypodynamic frog ringer over the heart. The inferior venacava was traced, put a thread around it and given a small cut in order to insert the venous cannula. The cannula was inserted in the vein and the thread was tied to assure the cannula in place which is in turn connected to a saline bottle containing hypodynamic frog ringer solution. A small cut in one of the aorta was given for the ringer to come out. Heart was isolated and attached to the stand with moderate flow of ringer. A thin pin hook was passed through the tip of the ventricle and with the help of a fine thread attached to the hook; it was tied to the free limb of the Sterling's heart lever which was fixed to a stand. A proper tension was adjusted by altering the height of the lever. The normal heart rate was noted. All test samples that were administered in different doses viz. 10,20,40,80 $\mu\text{g/ml}$ respectively. The rate and force of heart contraction were noted as given in (Table-3, Figure:6).

4. Results

Thin layer chromatography analysis of crude extract revealed 3 spots with Rf values 0.5,0.7,0.9. UV/Visible analysis of aqueous crude extract from aerial parts of *carmona retusa* was reported to have 2 Sharp peaks at 272.00, 666.00nm with absorption values 0.524, 0.182 respectively. FTIR spectrum results of crude extract were reported with 4 major peaks of which functional groups identified at 3309.24(OH), 2943.60 ($\text{CH}_2=\text{CH}_2$), 2831.71 (CHO), 1410.94(CH_3)

The results on cardiotoxic activity of *carmona retusa* are presented in Table-3, Figure 6. The results indicated that the extract on isolated perfused frog's hearts elicited dose dependent effect. There was an increase in force of contraction (the positive inotropic effect) and slight increase in cardiac output. The aqueous extract exhibit cardiac stimulant activity. The dose dependent changes were measured in heart rate (HR), cardiac output (CO) Height of force of contraction (HFC). In all these dose concentrations the extract with 80 $\mu\text{g/ml}$ exhibited prominent cardio tonic effect in hypodynamic heart.

Table 1: Proximate analysis of *carmona retusa*

Solvents	Aqueous Plant extract material	
	Ordinary light	UV light
Ethyl acetate	Dark green	Yellowish green
Distilled water	Brownish green	Yellowish green
1N NaOH	Brownish green	Yellowish green
1N HCl	Light green	Light green
50% H_2SO_4	Dark yellowish	Light yellowish

Table 2: Qualitative tests for phytochemical screening of crude aqueous extract

S. No	Test	Aqueous Extract
1	Carbohydrates	++++
2	Proteins	++
3	Steroids	++++
4	Alkaloids	++
5	Flavanoids	++++
6	Cardiac Glycosides	++++

++++; ++ Indicates higher & lower presence of bioactive compounds respectively.

Figure 2: Thin Layer Chromatography Studies on Aqueous crude extract of *Carmona retusa*



Figure 2a



Figure 3b

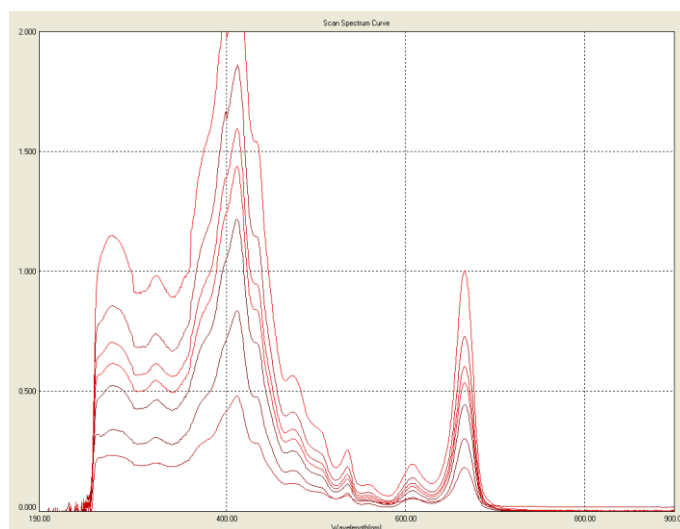


Figure 3: UV-Visible analysis data, Overlap spectra of 7 concentrations (5-30µg/ml) for the aqueous crude extract of *carmona retusa*.

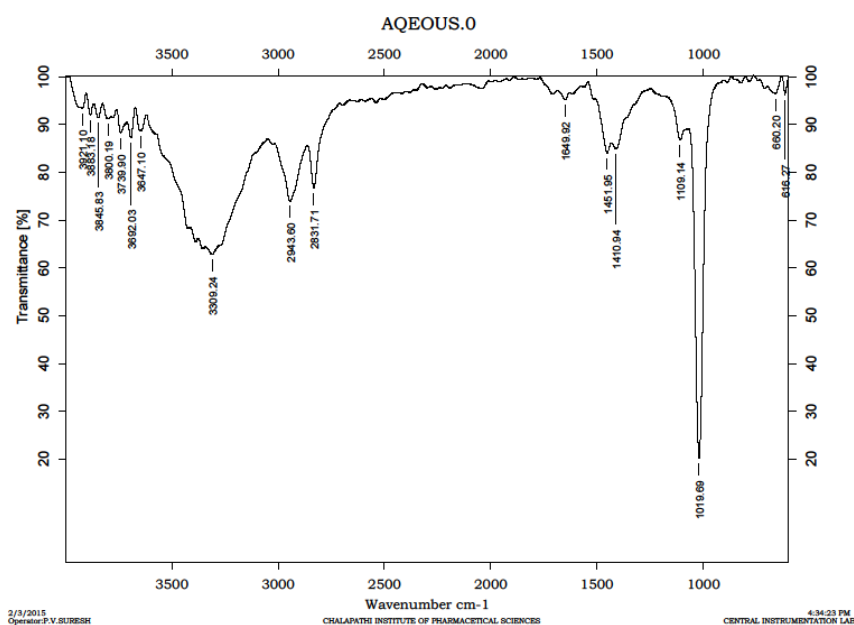


Figure 4: FTIR spectrum of aqueous crude extract of *carmona retusa*

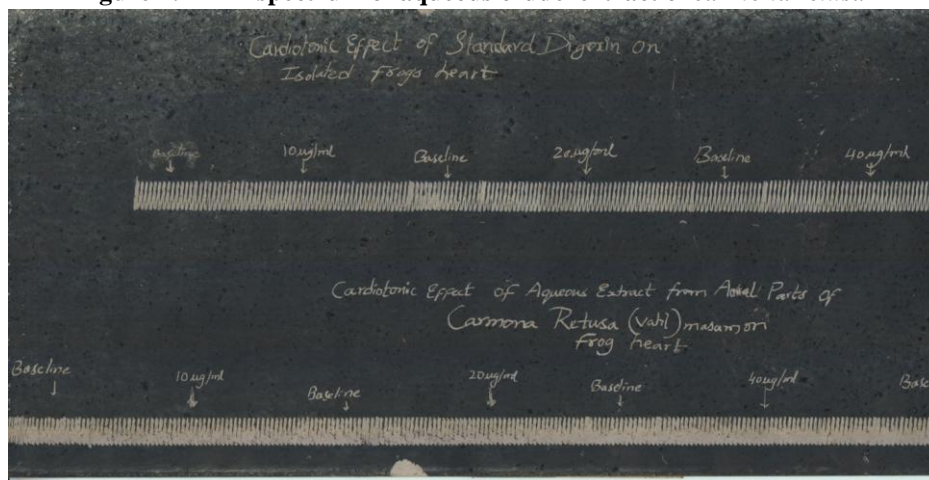


Figure 5: Cardiotoxic activity recorded on Kymograph for the aqueous crude extract

Table 3: Biological evaluation for cardiotoxic activity of aqueous extract of *Carmona retusa* (the parameters include the Height of force of contraction, heart rate and cardiac output.)

Drug Type	Conc (µg /ml)	Dose (ml)	Heart rate beats/min	HFC in mm	Cardiac output
Extract	10	0.1	46	0.7	32.2
	20	0.2	41	9	36.9
	40	0.4	38	1	38
	80	0.8	35	1.3	45.5
Digoxin	10	0.1	27	0.6	16.2
	20	0.2	17	0.9	15.3
	40	0.4	12	1.2	14.4
	80	0.8

5. Discussion

All the dilutions of *carmona retusa* restore cardiac activity of Hypodynamic frog heart i.e. it increases rapidity and force of contraction. It was found that greater the test dose concentration showed better response as compared to other doses. It is interesting to know that *carmona retusa* has rapid onset of action. These preliminary studies confirm the better cardiotoxic activity of *carmona retusa* and it can stand as better option next to Digoxin. Further studies can confirm the reduced toxicity & this will be the advantage of *carmona retusa* over digitalis.

6. Conclusion

Further extensive research is necessary to explore and isolate the active chemical constituents present in *carmona retusa* which are responsible for the cardiotoxic activity as well as to determine the possible mechanism of action.

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