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# CARDIO-VASCULAR ACTIVITY OF *GYNURA PROCUMBENS* MERR. LEAF EXTRACTS

Navneet Kaur*[1], Ammar Ihsan Awadh [1], Rabyah B. Ali [1], Amirin Sadikun [2], Munavvar Zubaid Bin Abdul Sattar [1] and Mohd Zaini Bin Asmawi [1]

Department of Physiology and Pharmacology [1], School of Pharmaceutical Sciences, USM, Minden, 11800 Penang, Malaysia
Department of Pharmaceutical Chemistry [2], School of Pharmaceutical Sciences, USM, Minden, 11800 Penang, Malaysia

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**Correspondence to Author:**

**Navneet Kaur**

(Ph. D. Student), Department of Physiology and Pharmacology, School of Pharmaceutical Sciences, USM, Minden, 11800 Penang, Malaysia

## ABSTRACT

*Gynura procumbens* has been known to be used as traditional medicine for treatment of kidney disease, rashes, fever and for hypertension. We report here the cardiovascular effects of the different ethanol (95%, 75%, 50%, 25% v/v) and water extracts using *in vitro* studies. *Gynura procumbens* leaves extract was prepared by maceration process. The cardiac effects (chronotropic and ionotropic) of each extract were examined on isolated right and left atrium which was prestimulated with $5x10^{-8}$ M Isoprenaline. Vasorelaxant effect was examined on intact isolated endothelium rat aorta rings precontracted with $10^{-6}$ M Phenylephrine. Water extract was found to be most potent to exhibit dose dependent vasorelaxation and negative chronotropic and ionotropic effects. Chemical analysis of GPWE showed the presence of significant amounts of polyphenolic and flavonoid constituents. The data suggests GPWE contains cardiovascular active substances which are responsible for significant dose-dependent cardiovascular effects. Such cardiovascular effects can possibly be attributed to the high polyphenolic content of this plant.

**INTRODUCTION:** Hypertension and other cardio-vascular diseases are the leading causes of morbidity and mortality worldwide. Globally, enormous efforts are being done to find the better therapeutic options for cardiovascular diseases. Traditional herbs have been widely used and served as major sources of drug research in pharmaceutical R&Ds. About 30% of worldwide sales of drugs are based on natural products [1].

*Gynura procumbens* Merr. is a fast growing herb belonging to family compositae, and is widely distributed in certain areas of Southeast Asia. The leaves of this plant are routinely used in Indonesia for the treatment of kidney diseases, eruptive fever, rash, hypertension, diabetes mellitus and hyperlipidemia [2].

It has been reported that an ethanolic extract of *Gynura procumbens* significantly reduces serum cholesterol and triglyceride levels of diabetic rats. Furthermore, the terpenes and saponins found in the organic extract of plant have been demonstrated to possess antiinflamatory activity. Antihypertensive effect of this plant has been reported [3], but plant cardiovascular effect have not been studied.

We report in this investigation, the cardio-vascular effects of different extracts of *Gynura procumbens* using *in vitro* experimental approach.

**MATERIALS AND METHODS:**

**Plant material**: *Gynura procumbens* Merr. was obtained from Penang Island, Malaysia. A voucher specimen 10833 was deposited at the Herbarium of the School of Biological Sciences, University Sains Malaysia.

**Preparation of Crude Extract**: The leaves were separated from the small branches of plant and dried in the hot air oven at the 40$^{o}$C for 3 days. The dried leaves were milled into a fine powder using a milling machine. The powdered leaves were extracted by maceration process at 60$^{o}$C in water bath using different concentration of ethanol i.e., 95%, 75%, 50%, 25%, and 0%v/v (water extract). These extracts were then concentrated on rotary evaporator (Buchi, Switzerland) under reduced pressure and subsequently freeze dried. The yield obtained for each extract was as follows:

| Extract Type | Description of Soild Extract Obtained | Yield |
|---|---|---|
| 95% Ethanolic Extract | A dark green wax like material | 6.4% |
| 75% Ethanolic Extract | A dark green hard material | 14.8% |
| 50% Ethanolic Extract | A dark dirty brown hard material | 18.7% |
| 25% Ethanolic Extract | A dark brown hard material | 22.1% |
| Water Extract | A light brown fluffy material | 28.7% |

**Drugs and solutions:** For extraction, ethanol was purchased from Fisher Scientific, UK. For preparation of Kreb's solution, Sodium Chloride, Potassium Chloride, Potassium Dihydrogen Phosphate, Magnesium Sulphate, Sodium Bicarbonate, Calcium Chloride and Glucose were purchased from R&M Chem., UK. Phenylephrine and Isoprenaline were purchased from Sigma-Aldrich, Germany. For determination of total flavonoids and total phenolic contents, Folin-Ciocalteu reagent, Gallic Acid and Quercetin were purchased from Sigma-Aldrich, Germany. While Sodium Carbonate, Aluminium Chloride and Potassium Acetate were bought from R&M Chem., UK. All the extracts were dissolved just before use in Kreb's physiological solution of following composition in mM: NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, Glucose 11.7

**Animals**: Experimental animals consisted of adult male SD rats 250-300g, which were bred and obtained from Animal Research And Service Centre (ARASC), USM, Malaysia. The animals were housed in standard environmental conditions (25$^{o}$C, 60-70% humidity) under natural lighting and fed with normal commercial rat chow (Gold Coin Feed Mills Sdn. Bhd., Malaysia) and water *ad libitum*. The rats were acclimatised for one week in transit room before used for experiments. Guidelines of the Animal Ethics Committee, USM, Malaysia, were followed while handling and taking out tissue from animals. For aortic ring experiments number of determinations were 8 rings, while for left and right isolated atrium number of determinations were 6 for each tissue.

**Aortic rings for tension measurement (Vaso Relaxation):** The rats were sacrificed in a carbon dioxide chamber (60 to 90 sec.). The thoracic aorta was rapidly removed, placed in a pertidish with Kreb's solution of following composition in mM: NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, Glucose 11.7 (aerated continuously with 95% $O_2$ + 5% $CO_2$, Carbogen) at room temperature, cleaned of fat and connective tissue, special care was taken to avoid damage to the endothelium. Cut into approx. 4-5mm long cylindrical segments.

Aorta rings were suspended horizontally in an organ bath containing 10ml of the above solution, thermoregulated at 37$^{o}$C and bubbled with carbogen with a resting tension of 1g. The rings were equilibrated at 1g resting tension for 60 min, during which the bathing solution was replaced every 10 min and if needed, the tension was readjusted to 1g. Isometric contraction and relaxation were recorded using Force Displacement Transducer (model- Grass® Force Displacement Transducer FT03). The transducer signals were displayed and stored on a computer for further analysis using AD Instruments Power Lab Software). Concentration dependent relaxation of extracts were studied by precontracting the rings with $10^{-6}$ M phenylephrine and when stable maximal contraction plateau was reached, cumulative concentrations (0.25, 0.5, 1.0, 2.0mg $ml^{-1}$ of organ bath) of extract were added at intervals of 15 min. Dose response curve of different extract were assessed by comparing their vasorelaxing response [4].

**Right atrium for beat per minute measurement (Chronotropic Effect):** The rats were anaesthised in carbon dioxide chamber (40 sec) and subsequently exanguinated. Carefully, heart was removed and

placed in a petri dish containing Kreb's solution aerated with carbogen. The spontaneously beating right atrium was isolated with precautionary steps to ensure no damage to SA node was done. Right atrium was suspended in organ bath containing 10 ml of physiological solution, thermoregulated at 37$^{o}$C and bubbled with carbogen, with a resting tension of 1g.

The right atrium was stabilised at 1g resting tension for 90min. During stabilisation the bath solution was replaced every 10 min and if needed the tension was readjusted to 1g. Heart rate (beats per min.) were recorded using Force Displacement Transducer (model Grass®FT03). The transducer signals were displayed and stored on a computer for further analysis using AD Instruments Power Lab Software. Concentration dependent chronotropic effect of extracts were studied by inducing maximum BPM with $5x10^{-8}$ M isoprenaline and when stable BPM plateau was recorded, cumulative concentration (0.25, 0.5, 1.0mg $ml^{-1}$ of organ bath) of extracts were added at intervals of 3 min. Concentration response curve of different extracts were assessed by comparing their chronotropic effects.

**Left atrium for amplitude of cardiac muscle contractile tension measurement (Ionotropic Effect**): From the above heart, left atrium was isolated and suspended in between electrodes to enable electrical stimulation (2Hz, 5ms, threshold voltage + 50%) (Grass®S6 stimulator) containing 10ml of Kreb's solution, thermoregulated at 37$^{o}$C and bubbled with carbogen with a resting tension of 1g. The left atrium was stabilised, at 1g resting tension, for 90 min and during stabilisation the bath solution was replaced every 10min. and if needed the tension was readjusted to 1g. Systolic isometric contraction and relaxation were recorded using force displacement transducer and signals were analysed using AD Instrument Power lab software. Concentration dependent ionotropic effect of extracts were studied by pre inducing maximum amplitude of contraction to the left atrium with $5x10^{-8}$ M isoprenaline and when stable contraction plateau was reached, cumulative concentration (0.25, 0.5, 1.0, 2.0mg $ml^{-1}$ of organ bath) of extract were added at intervals of 3 min. Dose response curve of different extracts were assessed by comparing cardiorelaxing effect of extracts [5].

**Chemistry**: Based on the experimental results, further attempts were made on the most potent plant extract to chemically define the composition of the active crude extract using the chemical analytical procedures below:

- **Determination of Total Phenolic Contents**: According to the method of Slinkard and Singleton (6) the total soluble phenolic contents in the most active plant extract were determined using Folin-Ciocalteu reagent and gallic acid as standard. A solution of 4mg $ml^{-1}$ of the plant extract in methanol and solution of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0mg $ml^{-1}$ of gallic acid in methanol were prepared. 20µl of the active plant extract and each concentration of gallic acid solution were pipetted in separate test tubes followed by the addition of 1.58ml of distilled water and 100µl of 2N Folin-Ciocalteu reagent. Subsequently, the test tubes were mixed thoroughly. After 8 min, 300µl of 20% sodium carbonate solution were added. The mixture was allowed to stand for 2hr with intermittent shaking. The absorbance of solutions was measured at 765nm with a Hitachi U-2000 spectrophotometer. The total phenolic compounds concentration in extract was determined in milligram of gallic acid equivalent by using an equation obtained from a standard gallic acid graph.

- **Determination of Total Flavonoid Content**: The total flavonoid contents in the most active plant extract were determined by aluminium chloride colorimetric method using quercetin as standard [7, 8]. Dissolve 4mg $ml^{-1}$ solution of active plant extract in methanol and solutions of 0.0078, 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0mg $ml^{-1}$ of quercetin in methanol were prepared. 500µl of the active plant extract and each concentration of quercetin solution were pipetted in separate test tubes followed by the addition of 0.1ml of 10% (w/v) aluminium chloride solution, 0.1 ml of 1M potassium acetate solution, 1.5 ml of methanol and 2.8 ml of distilled water. The test tubes were thoroughly mixed and after incubation at room temperature for 30min the absorbance of the reaction mixture was measured at 415nm with a Hitachi U-2000 Spectrophotometer.

The amount of 10% aluminium chloride was replaced by same amount of distilled water in a blank. The concentration of total flavonoid content in the extract was determined as microgram of quercetin equivalent by using an equation that was obtained from a standard quercetine graph.

- **Statistical Analysis:** All results were expressed as Mean ± Standard Error Mean per experimental groups and latter were analysed by one way analysis of variance (ANOVA). Dunnett's test was used to evaluate the significant differences between the control and the experimental groups. Differences with a $P<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION:

**Vasorelaxant effect of extract in rat isolated aorta ring**: Phenylephrine $10^{-6}$ M produced a maximum contraction of aortic ring. Cumulative addition of extracts (0.25, 0.5, 1.0, 2.0mg $ml^{-1}$) produced vasorelaxant effects in aortic rings precontracted with PE. Concentration of 0.25, 0.5, 1.0, 2.0mg $ml^{-1}$ *Gynura procumbens* water extract showed significant vasorelaxation as compared to other extracts. GPWE relaxed the precontracted aorta to the pre resting tension, which indicates the presence of high concentration of promising components in the water extract of *G. procumbens*.

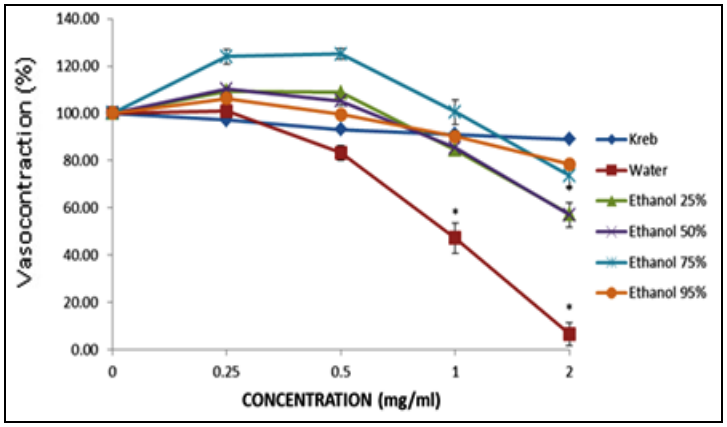


**FIG. 1: COMPARATIVE VASOCONTRACTION (%) EFFECT OF EXTRACTS (WATER, 25%, 50%, 75% AND 95% ETHANOL) ON THE AORTIC RINGS PRECONTRACTED WITH $10^{-6}$M PHENYLEPHRINE (PE).**

Data presented as Mean± SEM (n=8). *p<0.05, the effect of 1.0mg $ml^{-1}$ (water extract) and 2.0mg $ml^{-1}$ (water extract, 25% and 50% ethanol extract) of *Gynura procumbens* on vasorelaxation of PE precontracted aortic rings versus control. Data were analysed by one-way ANOVA followed by Dunnett post-hoc test.

**Chronotropic effect of extracts in rats isolated right atrium**: Isoprenaline $5x10^{-8}$ M produced maximum BPM (%) (beats per min.) in right atrium. Cumulative addition of extract (0.25, 0.5, 1.0, 2.0mg $ml^{-1}$) gave lowering of BPM (-ve chronotropic effect) in right atrium preinduced maximum BPM with isoprenaline. Maximum dose of extract given was 1mg $ml^{-1}$ as with higher concentrations atrium stopped beating. Out of five different extracts of *Gynura procumbens* water extract showed maximum lowering in BPM (-ve chronotropic effect). This result also indicates promising component in higher concentration is present in GPWE.

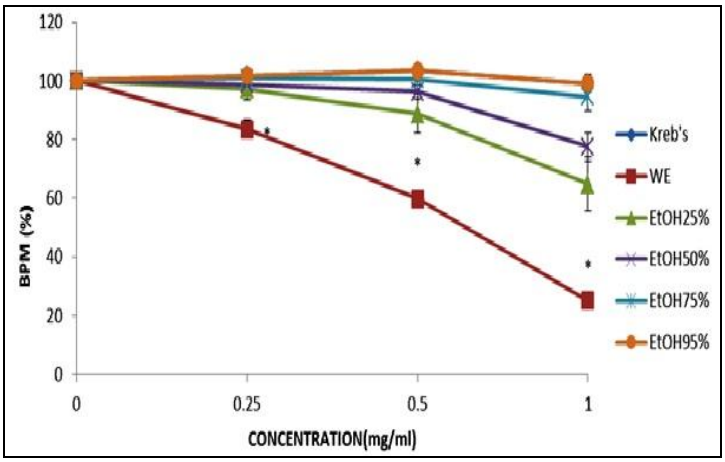


**FIG. 2: COMPARATIVE CHRONOTROPIC EFFECT OF EXTRACTS (WATER, 25%,50%, 75% AND 95% ETHANOL) ON THE RIGHT ATRIUM PREINDUCED WITH $5x10^{-8}$ M ISOPRENALINE**

Data presented as Mean± SEM (n=6). *p<0.05, the effect of 0.25mg $ml^{-1}$ (water extract), 0.5mg $ml^{-1}$ (water extract, 25% ethanol extract), and 1.0mg $ml^{-1}$ (water extract, 25% and 50% ethanol extract) of *Gynura procumbens* –ve chronotropic effect on IsoP preinduced right atrium versus control. Data were analysed by one-way ANOVA followed by Dunnett post-hoc test.

**Ionotropic effect of extracts in rats isolated left atrium**: Isoprenaline $5x10^{-8}$ M showed maximum strength of contraction of cardiac muscles. Cumulative addition of extracts (0.25, 0.5, 1.0, 2.0mg $ml^{-1}$) showed decrease in strength/force of contraction (-ve ionotropic effect) to the precontracted/induced left atrium with isoprenaline. Left atrium also stopped beating at higher concentrations >2mg $ml^{-1}$ of *Gynura* extracts, so could not study at higher concentrations. GPWE showed maximum reduction in strength/force of contraction of cardiac muscles (-ve ionotropic effect). It also points out promising compound in GPWE.

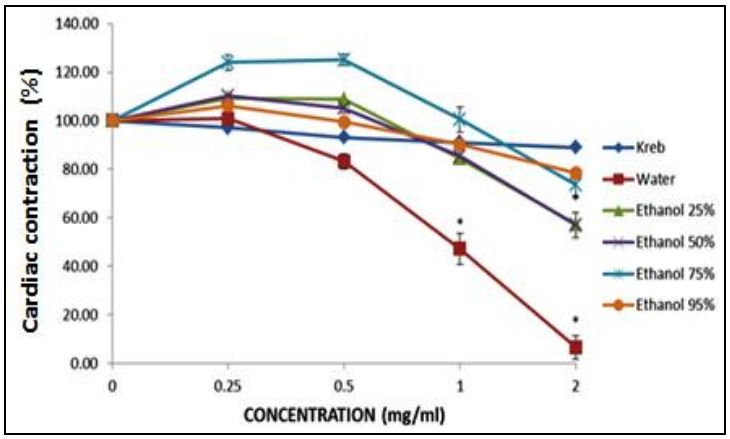


**FIG. 3: COMPARATIVE IONOTROPIC EFFECT OF EXTRACTS (WATER, 25%, 50%, 75% AND 95% ETHANOL) ON THE LEFT ATRIUM PRECONTRACTED WITH 5x10$^{-8}$M ISOPRENALINE**

Data presented as Mean ± SEM (n=6). *p<0.05, the effect of 0.25mg ml$^{-1}$ (water extract), 0.5mg ml$^{-1}$ (water extract, 25% ethanol extract), and 1.0mg ml$^{-1}$ (water extract, 25% and 50%, 75%, 95% ethanol extract) of *Gynura procumbens* veionotropic effect on IsoP precontracted right atrium versus control. Data were analysed by one-way ANOVA followed by Dunnett post-hoc test.

**Chemistry**: The results of the vasorelaxation, ⁻ve chronotropic and –ve ionotropic effects of GPWE forced the interest to look deeper into the chemical composition of this extract and possible constituents responsible for these effects. The calculated phenolic contents of GPWE were found to be 12.5%, While the total flavonoid contents of the same extract were approx. 1.6%. Flavonoids comprise a large group of naturally existing polyphenolic compounds widely distributed throughout the plant kingdom. These compounds are reported to have cardiovascular effects and contribute to an essential reduction in the occurance of cardiovascular diseases [9].

In summary, the *in vitro* vasorelaxation, chronotropic and ionotropic experiments suggests that GPWE is the promising extract among all the extracts obtained from *G. procumbens* to produce concentration dependent relaxation of vascular smooth muscles, ⁻ve chronotropic effect and –ve ionotropic effect in the isolated aorta, right atrium and left atrium respectively. GPWE possesses the ability to dilate the vessels along with reduction in heart rate and force of contraction of heart. As the data shows, -ve values indicating non-competitive effects.

This finding indicates the presence of biologically active constituents that can be isolated, purified and used for cardiovascular ailments. Moreover, these findings justify the traditional use of this plant in hypertension management.

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