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Antihypertensive Potency of Wild Cosmos (*Cosmos caudatus* Kunth, Asteraceae) Leaf Extract

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ABSTRACT

The wild cosmos (*Cosmos caudatus* Kunth, Asteraceae) is one of the vegetables mostly consumed by rural people of Central and East Java Provinces of Indonesia and those of the Malay Peninsula. Yet, it has not been widely used medicinally. The leaf has distinctive taste as well as odor and contains high level of flavonoid, especially flavonol and flavon glycosides which have potent antioxidant activity. In this study, in an attempt to explore the antihypertensive effect, wild cosmos leaf aqueous extract was tested in rats treated with adrenaline and sodium chloride. The frequency of heart rate and amplitude of stroke volume were measured using the non-invasive tail cuff method. The extract at doses of 500 and 1000 mg kg^{-1} showed similar potency to that of 9 mg kg^{-1} atenonol, in lowering both parameters, induced by adrenaline. However, after sodium chloride, the extract only suppressed the amplitude and this effect was comparable to that of 0.45 mg kg^{-1} hydrochlorothiazide and 13.5 mg kg^{-1} captopril. Besides cardiac effects, the extract also demonstrated diuretic activity which was comparable to that of 1.8 mg kg^{-1} furosemide. Taken together, results of present study suggest that wild cosmos leaf extract have antihypertensive effect which may be related, at least in part, to the decreased cardiac output and induction of diuresis. The results may further indicate that consumption of wild cosmos leaf in diet is beneficial for hypertensive patients.

Key words: Antihypertensive, wild cosmos, *Cosmos caudatus*, aqueous extract, leaf

INTRODUCTION

Wild cosmos (*Cosmos caudatus* Kunth, Asteraceae), known locally in Indonesia as Kenikir, is a common vegetable served at meal tables, generally freshly consumed as salad. In East Java Province of Indonesia, the plant is commonly used to help reduce the high blood pressure. Meanwhile, being known as ulam raja, in the region of Malay Peninsula wild cosmos has been traditionally used to improve blood circulation (Shui *et al*., 2005; Andarwulan *et al*., 2010). Previous study have revealed that the plant had high content of antioxidant. Indeed, in 100 mg of fresh sample the antioxidant capacity was equivalent to 2400 mg L^{-1} ascorbic acid (Shui *et al*., 2005). More recent work has shown that the extract of *C. caudatus* was the highest to contain flavonoid among nine aqueous extracts of Malaysian plants tested (Sumazian *et al*., 2010). This data was corroborated by previous results which demonstrated that aquesus extract of *C. caudatus* leaf had high content of flavonoid (Sukrasno *et al*., 2011). Flavonoid is

well-known for its potent antioxidant and radical scavenging activities that might be potential for preventing degenerative diseases (Tabak *et al*., 2001; Cibin *et al*., 2006; Naseri *et al*., 2008; Oloyede *et al*., 2011).

Despite the traditional use of wild cosmos extract for ameliorating the cardiovascular system performance, specifically in lowering the hightened blood pressure, so far no controlled studies on antihypertensive effect of wild cosmos extract has been reported. In this study the effect of the aqueous extract of wild cosmos leaf was tested on the frequency of heart rate and amplitude of stroke volume which are components of cardiac output, using the non-invasive tail cuff method in rats, induced by adrenaline or sodium chloride. In addition, diuretic effect of the extract was also tested.

MATERIALS AND METHODS

Animal and plant materials: Male Wistar rats (supplied by the Laboratory of Animal and Biological Test of School of Pharmacy, Institute of Technology Bandung) weighing 200-250 g were used. They were kept at constant ambient temperature of 23±2°C under a 12 h light/dark cycle with free access to food and water except during experimental procedures. Young leaf of *Cosmos caudatus* Kunth, collected from local growers in Eastern Bandung, was dried by aeration at room temperature. The dried material was then ground to produce coarse powder. The experiments were carried out from January to May 2010.

Extract preparation: The extract was prepared by boiling 200 g of the ground wild cosmos leaf in 1 L of distilled water for 30 min and filtered through Whatman filter paper. The residue was re-extracted twice, each with 200 mL distilled water. Pooled extracts were then freeze dried.

Effect of wild cosmos leaf extract in adrenaline-treated rats: The rats were assigned into six groups of six. Each group was treated with oral vehicle, 9 mg kg^{-1} atenolol or extract of either of 250, 500 or 1,000 mg kg^{-1}. Measurements of frequency of heart rate and amplitude of stroke volume were performed before drug treatment (T-0), 60 min after drug (T-1) and 4 min after induction with intraperitoneal adrenalin at 1.2 mg kg^{-1} (T-2). These measurements were carried out non-invasively using tail-cuff method, with similar apparatus previously applied elsewhere (Rocha *et al*., 2008).

Effect of wild cosmos leaf extract in sodium chloride-treated rats: The rats were assigned into seven groups of six, treated either with oral vehicle, 13.5 mg kg^{-1} captopril, 0.45 mg kg^{-1} hydrochlorothiazide, 250, 500 or 1000 mg kg^{-1} extract. Sodium chloride (3.75 g kg^{-1} orally) induction was conducted for 14 days and continued for the following 14 days co-administered with drug treatments. The frequency of heart rate and amplitude of stroke volume were measured prior to sodium chloride induction, on day 15 during the daily sodium chloride treatment and day 28 after the induction (after 14 days of daily drug treatment).

Diuretic test: Diuretic test was conducted as described by Lipschitz and Stokey (1984) with a minor modification using metabolic cage. Rats were assigned into one of five groups of six rats, treated either with oral vehicle, 1.8 mg kg^{-1} furosemide, 250, 500 or 1,000 mg kg^{-1} extract. The rats

were fasted for 18 h and immediately before the experiment, a loading dose of warm water at 50 mL kg^{-1} was administered to each rat. Urine was collected and the pooled volume was measured every hour for 6 h and at the 24th hour.

Statistical analyses: In the statistical analyses, one-way ANOVA was used to compare means of the parameters at different observation times for each treatment and Fisher's Least Significant Difference (LSD) tests were used for *post hoc*. A difference was considered significant at $p<0.05$.

RESULTS

Effect of wild cosmos leaf extract on adrenaline-treated rats: As presented in Fig. 1, rats in group receiving no adrenaline treatment did not show different heart rate frequency in each measurement, while those treated with adrenaline only demonstrated significant increase in the frequency at T-2 compared to T-0 and T-1 (2.88±0.19 vs. 1.59±0.10 and 1.71±0.15 Hz, $p<0.0001$). The frequencies in rats treated with atenolol as well the extract at 500 and 1000 mg kg^{-1} at T-2 were not significantly different compared to those measured at T-0 and T-1 (1.72±0.18 vs. 1.58±0.11 and 1.64±0.23 Hz, 1.83±0.21 vs. 1.73±0.15 and 1.67±0.18 Hz, 1.76±0.20 vs. 1.62±0.17 and 1.71±0.23 Hz, respectively). This data indicates the preventing effect of the test substances on the adrenaline-induced increase in heartbeat frequency. At T-2, the heart rate frequency of rats treated with extract at dose of 250 was significantly higher compared to the frequency at T-0 and T-1 (2.11±0.20 vs. 1.58±0.11 and 1.76±0.09 Hz, $p<0.0001$), showing the inability of the extract at this dose to counter adrenaline effect on heart rate frequency.

In terms of stroke volume amplitude (Fig. 2), the results showed that atenolol and the extract at 500 and 1000 mg kg^{-1} prevented the adrenaline-induced elevated amplitude. In these groups, there were no significant differences in the amplitude before and after adrenaline induction (21.69±1.39 vs. 21.88±2.26 and 21.52±1.55×10^{-2} mv, 22.05±0.46 vs. 22.58±1.31 and 22.25±0.52×10^{-2} mv, 22.00±0.97 vs. 22.07±0.37 and 22.24±0.87×10^{-2} mv, respectively). These results were not significantly different from those observed in group without adrenaline induction (21.58±0.96 vs. 20.98±1.85 and 21.76±0.96×10^{-2} mv). The group treated with 250 mg kg^{-1} extract,

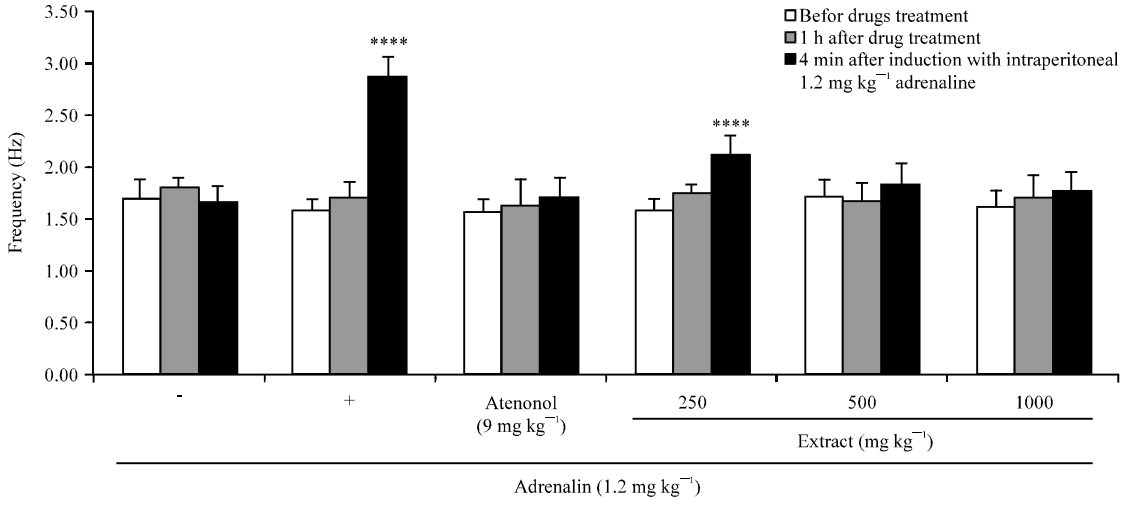

Fig. 1: Effect of wild cosmos leaf extract on adrenalin-induced increase in heart beat frequency in rats. Data averaged from 5 rats, ****$p<0.0001$ vs. pre-treatment group (one-way ANOVA, followed by Fisher's Least Significant Difference test)

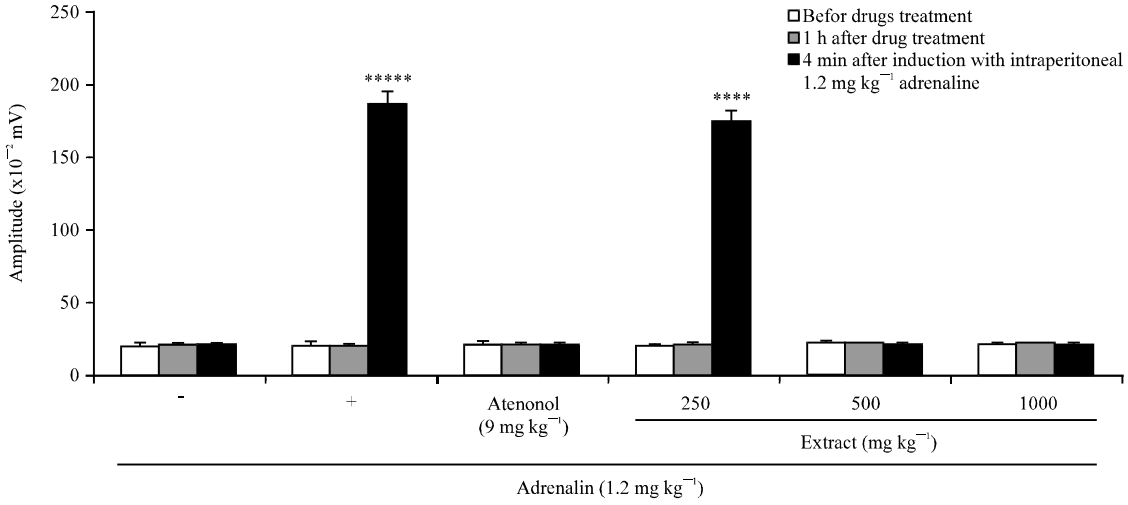

Fig. 2: Effect of wild cosmos leaf extract on adrenalin-induced increase in stroke volume amplitude in rats. Data were average from 5 rats, ****p<0.0001 vs. pre-treatment group (one-way ANOVA, followed by Fisher's Least Significant Difference test)

on the other hand, did not show suppression in the amplitude (186.98±8.20 vs. 20.85±2.68 and 20.21±0.34×10^{-2} mv, p<0.0001, respectively), as also found in adrenaline only-treated group (174.5±57.68 vs. 20.28±1.21 and 21.46±0.98×10^{-2} mv, p<0.0001).

Effect of wild cosmos leaf extract on sodium chloride-treated rats: As shown in Fig. 3, there was no significant difference in heart rate frequency in rats receiving repeated sodium chloride at 3.75 g kg^{-1} as compared to the non-treated rats or to that before the drug treatments. In addition, captopril or hydrochlorotiazide treatment for 14 days, following repeated treatment with sodium chloride, also failed to induce differences in the frequency compared to that before the drugs treatment. Administration of sodium chloride at 3.75 g kg^{-1} for 14 days, on the other hand, led to significant differences in stroke volume amplitude in rats receiving no drug treatment (value on day 0, 14 and 28 was 22.20±2.73, 147.11±16.71 and 163.38±24.64×10^{-2} mv, respectively). Treatments with hydrochlorotiazide, captopril, 500 and 1000 mg kg^{-1} extract suppressed the sodium chloride-induced increased amplitudes (values on day 14 vs. 28 were 146.12±22.19 vs. 21.70±3.08, 148.50±18.91 vs. 22.97±3.42, 180.90±17.56 vs. 23.84±1.68, 204±11.39 vs. 23.62±1.25×10^{-2} mv, respectively). Such finding was not observed after treatment with 250 mg kg^{-1} extract (the amplitude on day 0, 14 and 28 was 19.74±4.26, 192.57±20.21 and 206.57±17.02×10^{-2} mv, respectively).

As depicted in Fig. 4, all tested drugs failed to inhibit sodium chloride-induced increase in stroke volume amplitude on day 15 during the daily sodium chloride treatment. From an average value of 25×10^{-2} mv prior to sodium chloride treatment, the amplitude significantly increased to as high as 200×10^{-2} mv. However, on day 28, the amplitude in groups receiving reference drugs as well as the extract at 500 and 1000 mg kg^{-1} returned to the normal level.

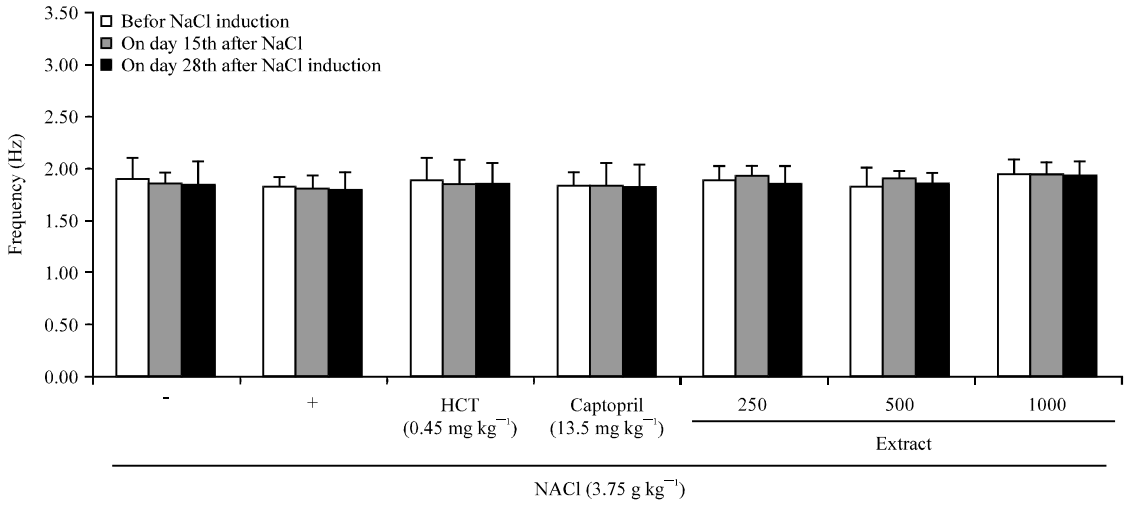

Fig. 3: Effect of wild cosmos leaf extract on the heart rate frequency of sodium chloride-treated rats. Data were average from 5 rats. HCT: Hydrochlorothiazide

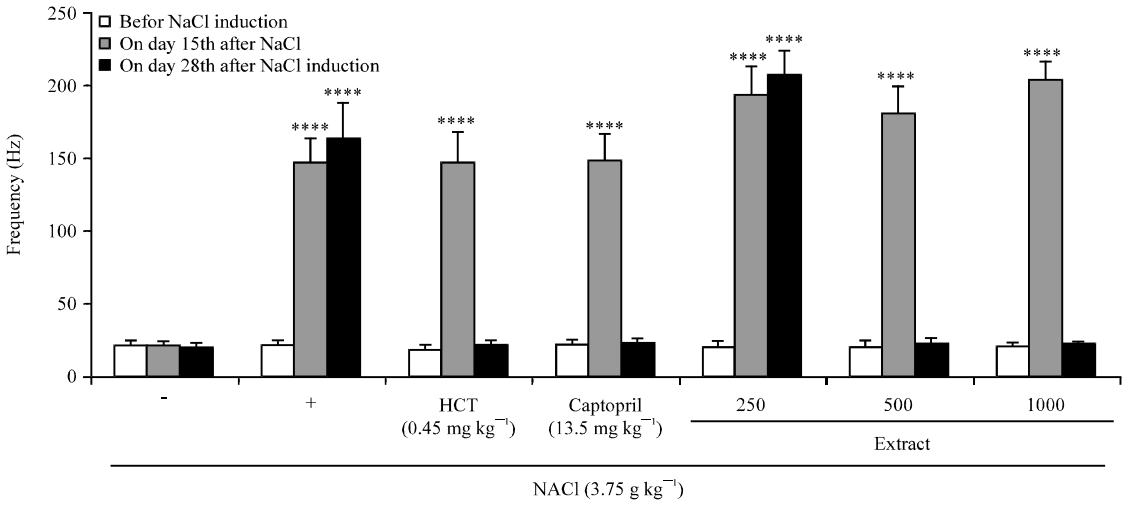

Fig. 4: Effect of wild cosmos leaf extract on sodium chloride-induced increase in stroke volume amplitude in rats. Data were average from 5 rats, ****p<0.0001 vs. pre-treatment group (one-way ANOVA, followed by Fisher's Least Significant Difference test). HCT: Hydrochlorothiazide

Diuretic effect of wild cosmos leaf extract: Results of diuretic tests were presented in Fig. 5. The rats were fasted for 18 h and immediately before the experiment, a loading dose of warm water at 50 mL kg^{-1} was administered to each rat. Urine was collected and the pooled volume was measured every hour for 6 h and at 24th h. Data were average from 5 rats (blank, dotted, close dotted, lined and filled column represents vehicle, 1.8 mg kg^{-1} furosemide, 250, 500 and 1,000 mg kg^{-1} extract, respectively). Pooled volume of urine during the first hour was increased significantly after the administration of 1000 mg kg^{-1} extract (pooled volume was 2.83±0.26 in

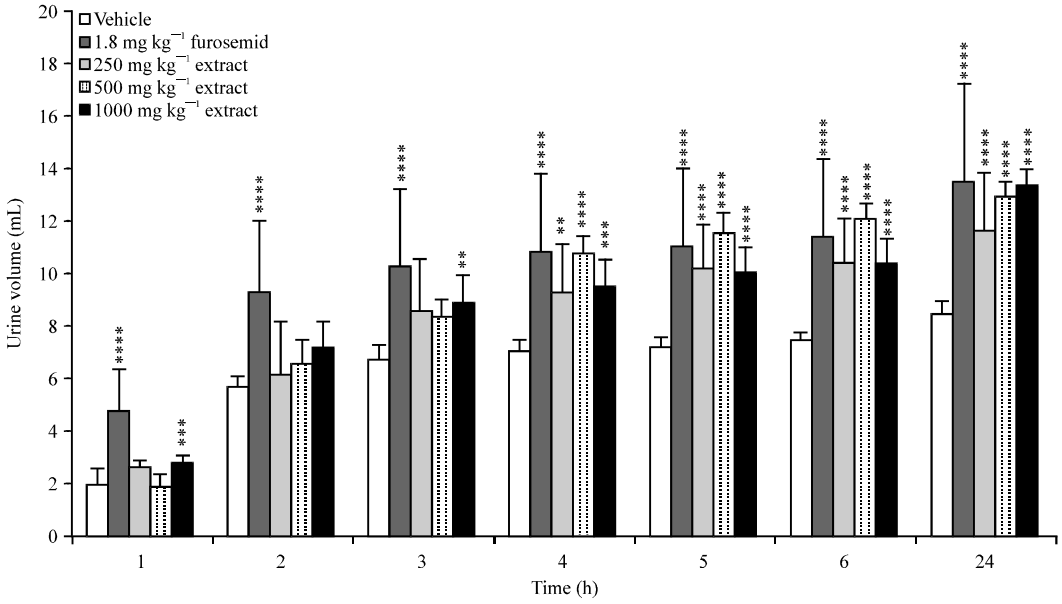

Fig. 5: Diuretic effect of wild cosmos leaf extract in rats, **,***,****p<0.01, 0.001 and 0.0001, respectively vs. vehicle group (one-way ANOVA, followed by Fisher's Least Significant Difference test)

extract group and 4.75±1.61 in furosemide group vs. 2.00±0.55 of the control group, p<0.0001 and p<0.001, respectively). It was not until the fourth hour of the observation period that the increases in pooled volumes in extract-treated groups were persistent. After 24 h, the extract was shown to increase the pooled volume in a dose dependent manner and the effect was comparable to that exerted by furosemide (pooled volume for furosemide, 250, 500 and 1000 mg kg^{-1} extract was 13.50±3.76, 11.67±2.18, 12.92±0.58, 13.33±0.68, respectively, statistical significance was found at p<0.0001 in all cases).

DISCUSSION

Results of the present study showed that aqueous extract of wild cosmos leaf at 500 and 1000 mg kg^{-1} blocked the increase in both frequency of heart rate and amplitude of stroke volume induced by adrenaline, while only demonstrated the action on the amplitude after sodium chloride induction. The results further revealed diuretic activity of the extract.

Adrenaline has been known as having both positive chronotropic and inotropic characteristics. The chronotropic effect of adrenaline in primary cardiac muscle cells has since been long documented to be attributed to the increase in the rate in diastolic depolarization (Hutter and Trautwein, 1956; West *et al.*, 1965). The results found after sodium chloride induction was interesting, in that it only induced the increase in stroke volume amplitude. This phenomenon might be attributable to negative chronotropic effect of hyperosmotic sodium chloride solution as reported in a number of studies (Bassani *et al.*, 1987, 1990). The hyperosmotic state might be achieved in the present study after repeated treatment of sodium chloride at a fairly high dose of 3.75 mg kg^{-1} which is in fact the oral LD50 of the substance in rat (Ash and Ash, 2004). Rhee *et al.* (1993) have shown that stroke volume index was increased significantly with decreased

heart rate, showing a reciprocal association between the two indices. Indeed, as found in the present study, an increase in heart rate frequency did not parallel the increase in stroke volume amplitude after sodium chloride treatment.

Cumulative data so far indicated that antioxidants have ameliorating effect on elevated blood pressure. As reported previously, *Cosmos caudatus* is a flavonoid-rich plant and among others, flavonoid has been well known to have potent antioxidant effect. There has been a number of evidence indicating the close association between oxidative stress as well as reactive oxygen species and cardiovascular diseases (Engelhard *et al*., 2006; Shinke *et al*., 2007; Victor *et al*., 2009a, b). Wild cosmos leaf extract used throughout the current study was obtained by boiling the crude drug in distilled water, based on the finding that flavonoids present in crude drug are mainly in the form of water-soluble glycosides. The extract is better prepared from fresh drug (not the dried form) since, as previously observed, boiling of fresh leaf yielded higher content of flavonoid (data not shown). Furthermore, drying temperature has been shown to affect the flavonoid content, with the optimum temperature to yield the highest flavonoid content to be 40°C. Indeed, drying the crude drug under higher temperature up to 60°C decreased the flavonoid content (Sukrasno *et al*., 2011). In practice, improvement in crude drug preparation may lead to reduction of the effective dose.

In view of the cardiac function, early data have demonstrated cross talk between the redox system and cardiac muscular activities. Thus, myocardial function can be altered by free radical generation, as shown by the impairment of cardiac function that occurs in relation to ischemia/reperfusion (Bolli *et al*., 1995). In addition, free radicals may play a role as tonic modulators of cardiac function in the setting of congestive heart failure (Ekelund *et al*., 1999) which is a model of chronic oxidative stress (Mak and Newton, 2001). Free radical activity may affect cardiac function by multiple possible mechanisms, including peroxidation of lipid membranes with consequent perturbation of membrane-bound enzymes and receptors (Williams *et al*., 1995), with ensuing events related to contractile activity (Rowe *et al*., 1983; Kim and Akera, 1987; Kaneko *et al*., 1989). In addition, it has been demonstrated that free radicals alter **β**-adrenergic receptor function and postreceptor signal transduction (Tan *et al*., 1995; Persad *et al*., 1997, 1998). All this evidence was later confirmed clinically in a study by Mak *et al*. (2000), showing that vitamin C, a well-known antioxidant, augment the left ventricular systolic pressure as well as heart rate in patients treated with dobutamine, a positive chronotrope/inotrope. While the aforementioned association seems to be close, it is interesting to note that the characteristic is contradictory to the present results. Instead of augmented, wild cosmos leaf extract decreased the effect of adrenaline, other well-known positive chronotrope/inotrope. Altogether, the data implicated that the active ingredient(s) of wild cosmos leaf other than antioxidant might be responsible for the dampening effect on cardiac output; alternatively, there was another distinct machinery instead of those mentioned above which served as pathway of the antioxidant's action. In this regard, a previous result showed that an extract from a plant rich in flavonoid induced no-mediated vasodilation (Amalia *et al*., 2008) which, in turn, affect peripheral resistance, the other determining factor of blood pressure. Further studies are therefore needed to clarify more exact mechanism of action of wild cosmos leaf extract component in this respect.

Additional data from diuretic activity study of the extract was encouraging. At 24 h the pooled urine volumes in the extract-treated groups were comparable to that treated with furosemide. This activity can provide additional benefit to the action of the extract on the cardiac function, as discussed above which could eventually lead to decrease in blood pressure. Although the exact mechanism in this regard has yet to be confirmed, the abundant flavonoid content of the extract

might take an important part. It was only recently that Junior *et al.* (2011) demonstrated in simultaneously hypertensive rats, using a bio assay-guided phytochemical study, the diuretic as well as potassium-sparing effect of quercitrin, the active flavonoid from *Tropaeolum majus*.

CONCLUSION

Results of the present study suggest that Cosmos leaf extract has the potency to decrease cardiac output, as indicated by its capacity to prevent harmful effect of adrenaline as well as sodium chloride on heart rate frequency and stroke volume amplitude. The extract also presented additional activity of inducing diuresis which can be synergistic to the reduction of blood pressure. The present findings may, therefore, lay a foundation for the development of potent alternative antihypertensive agents.

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