Vasodilator effect of the methylene chloride/methanol extract of Erythrina indica Linn (Leguminosae)

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ABSTRACT

Erythrina indica (Leguminosae) is used in folk medicine as a remedy for hypertension. In this study, the pharmacological activity of the methylene chloride/methanol (1 :1) extract of *E. indica* was determined in isolated rat thoracic aorta. The relaxant effects of *E. indica* on vascular preparation from rat aorta precontracted with norepinephrine or high concentration of KCl was concentration-dependent and endothelium-independent. Pretreatment of aortic rings with L-NAME (10^{-4} M) did not affect the relaxant effect of the plant extract at the higher concentrations of 400 and 800 µg/mL. Indomethacin (10^{-4} M) significantly reduced (-31% $p < 5\%$, with 100 µg/mL of extract) the relaxant effects of *E. indica* in endothelium intact arteries. These results indicate that the vasorelaxation effect of *E. indica* extract may be mediated at least in part by prostacyclin.

Key words : *Erythrina indica*, aorta, endothelium, vasorelaxation

RESUME

Effets vasodilatateurs de l'extrait au chlorure de méthylène/méthanol de *Erythrina indica* Linn (Leguminosae). *Erythrina indica* (Légumineuse) (E.i.) est utilisée en médecine traditionnelle comme remède contre l'hypertension artérielle. L'activité pharmacologique de l'extrait au chlorure de méthylène/méthanol de cette plante a été évaluée sur l'aorte thoracique isolé du rat. Les effets vasorelaxants de *Erythrina indica* sur des fragments vasculaires aortique précontractés par la noradrénaline ou par une forte concentration de KCl ont été concentration dépendent et endothélium-indépendants. Le traitement préalable des anneaux aortiques avec du N^{w}-nitro-L-arginine méthyl ester (L-NAME : 10^{-4} M) n'a pas affecté les effets relaxants de l'extrait de la plante à de fortes concentrations (400 et 800 µg/mL). L'indométhacine (10^{-4} M) a réduit de manière significative (-31 %, $p<5\%$ pour 100 µg/mL d'extrait) les effets relaxants de *Erythrina indica* sur les fragments artériels avec endothélium. Ces résultats montrent que les effets vasodilatateurs de l'extrait de *Erythrina indica* serait médiés, même en partie, par la prostacycline.

Mots clés : *Erythrina indica*, aorte, endothélium, vasorelaxation.

INTRODUCTION

It is well documented that several plants species have been used traditionnally in Africa and elsewhere for a variety of ailments. Tropical plant species are a natural resource for bioactive principles ; some of which play an important role in therapy and/or curative against pathogenic organism (Sofowora, 1983). In Africa, the exploitation of the medicinal plant has become a vital necessity in view of the escalating costs of synthetic drugs. Therefore, there is a critical need to identify, evaluate and preserve these vast plant resources before they are completely destroyed by deforestation and over exploitation.

Erythrina indica Linn. Syn. *E. variegata* Linn (Leguminosae) is a showy, spreading tree legume with brillant red blossomes occuring in the tropical and subtropical regions over the world (Dyke and Quessy, 1981). It is a medium to large tree, commonly reaching 15 to 20 m in height with an erect, spreading form, typically with several vertically oriented branches emerging from the lower stem (Hegde, 1993). The main active constituents of *Erythrina* species are alkaloids (Chawla et al. ; 1988, Nkengfack et al., 2001) and flavonoids (Tanaka et al., 2000). Most of them contain hypaphorine, erythraline, erysopine, eryvarin A and B, erysotine, erysovine, 3-phenyl coumarin, robustic acid, 8-prenyldaidzein, daidzein, cajanin and dimethyl alpinumisoflavone (Azebaze et al., 2000 ; Tanaka et al., 2000).

The root bark is employed in Cameroonian folk medicine for the treatment of trachoma, elephantiasis and microbial infections (Ayensu, 1978). The stem bark is used in decoction form as a laxative, diuretic and expectorant. The juice from the leaves is mixed with honey to stimulate lactation and menstruation (Hegde, 1993). Uses in therapy of hypertension (Chatterjee et al., 1981) and inflammation (Cox, 1993) are also reported. Although ***E. indica*** bark is used in Cameroon for the traditionnal management of hypertyension ; the plant is not cited in the national ethnobotanical review of Cameroon medicinal plants carried out by Adjanouhoun et al. (1996). Generally, substances that induces a decrease in blood pressure act via several vasodilatory mechanisms (Kimura et al., 1986 , Kazda et al ., 1988).

In the present work we have evaluated the effect of the stem bark methylene chloride/methanol extract of *E. indica* on the contraction of rat aortic muscle.

MATERIALS AND METHODS

Materials

Animals

Male Wistar rats (250 –350 g) raised in the animal house of the Faculty of Science, University of Yaounde I were used. The were fed a standard laboratory diet (S.P.C. Ltd, Bafoussam, Cameroon) and given fresh water ***ad libitum***.

Drugs.

Norepinephrine, indomethacin and L-NAME, were purchased from sigma Chemical Company (St. Louis, MO, USA). Ascorbic acid (0.57 mM) was added to each solution of NE, made up freshly every day. Indomethacin and N^{w}-nitro-L-arginine methyl ester (L-Name) were prepared as aqueous solutions and diluted with the bathing solution.

Plant material

Stem bark of *E. indica* was collected in June 1998 at Ibadan (Nigeria). A voucher specimen (N° 49028 HNC) documenting the collection was identified at the National Herbarium, Yaounde, Cameroon and is on deposit there. The stem bark was sun-dried and ground into powder. Then, air dried powdered stem bark of *E. indica* (6 kg) was extracted with 10 L of a mixture of methylene chloride/methanol (CH_2Cl_2/MeOH 1/1) and concentrated to dryness on a rotary evaporator under reduced pressure to afford a viscous mass of CH_2Cl_2/MeOH extract (250 g), with an extraction yield of 4.16%. Five gramme of this extract were dissolved in 1 mL of dimethyl sulfoxide (DMSO) and the solution adjusted to 100 mL with distilled water to obtain a stock solution of 50 mg/mL. Further dilution was made in Physiological salt solution (PSS). The final DMSO concentration did not produce significant effect on contractile responses.

Experimental methods

Tissue preparation and experimental protocol.

Experiments were performed on the isolated rat thoracic aorta as described previously (Dimo et al., 2002). Male *wistar* rats (250 - 350 g) were killed by decapitation and their thoracic aortas were removed and placed in modified Krebs buffer solution containing in mM ; NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11.1. The aortas were dissected from surrounding tissues and cut into rings approximately 3 - 4 mm wide. The endothelium was kept intact in some rings, but in another group of ex-

periments, the endothelium was removed from the aortic ring by rubbing the luminal surface with cotton thread. The aortic rings were suspended horizontally between two stainless steel wire hooks in organ chambers filled with 5 ml of physiological salt solution (PSS) 37° C, pH 7.4 and bubbled continuously with a mixture of 95% O_2, 5% CO_2. One wire was anchrored to the organ chamber plastic holder and the other was connected to a transducer couple Narco bio-system for the recording of the isometric tension (Bonnet et al., 1992). Preparations were submitted to a basal tension of 1 g and were allowed to equilibrate for 60 min., during which the bath solution was renewed every 15 min. Then, endothelium integrity was functionally assessed by evaluating the ability of acetylcholine (Ach., 10^{-5} M) to produce relaxation of preparations precontracted with norepinephrine (NE, 10^{-8} M). Preparations were considered to contain a viable endothelium when Ach. evoked relaxations exceeding 64% of precontraction, and were considered to be endothelium denuded when Ach. failed to cause relaxation (Furchgott and Zawadski, 1980). After Ach. testing, the aortic rings were washed with PSS three times during the next hour, prior to the next sequence.

Following the equilibration period, cumulative concentration-response of rings with and without endothelium were studied by contracting them with NE (10^{-6} M) or KCl (60 mM) for 30 minutes and then allowing them to relax in the presence of *E. indica* extract. Only one agonist was used in each experiment. When the contractile response to each agonist was stable, *E. indica* was added in progressively increasing cumulative concentrations. Rings were allowed to reach a new steady-state tension before each successive addition of the extract.

In the second group of experiments, tissues containing an intact endothelium were incubated for 30 min. with N^{w}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor or indomethacin (10^{-4} M) to prevent production and release of prostacyclin and a cumulative concentration-response of the extract was tested.

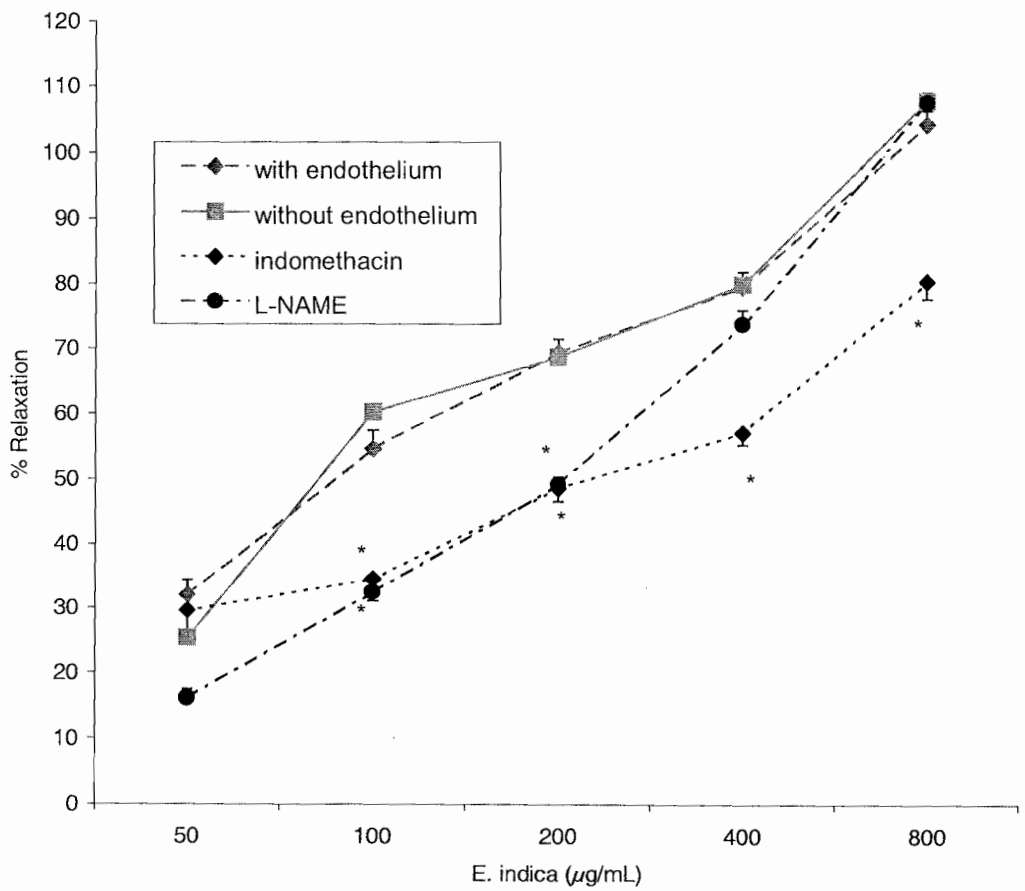

Fig. 1 : Concentration-response curves for relaxant effect of *E. indica* on the contractions induced by norepinephrine in endothelium intact and denuded aortic rings and in the presence of indomethacin and L-NAME. Data are presented as means ± S.E.M. (n = 5). *$p < 0.05$ as compared with the control.

Expression of results and statistical analysis.

Data are expressed as mean ± standard error of the mean (SEM), n representing the number of rats used in each experiment. Changes in aortic tension were expressed in percentage of either NE-induced or KCl-induced tension. The IC_{50} values were calculated as the *E. indica* concentration that cause a 50% of maximal relaxation (E_{max}). The one way analysis of variance (ANOVA) was used to determine statistical significance of differences between treatments ; $p < 0.05$ was considered to be statistically significant.

RESULTS

The relaxant effects of *E. indica* on vascular preparations from rat aorta precontracted with NE or high concentration of KCl are shown in Fig. 1 and 2. In all preparations investigated, *E. indica* induced relaxation of the precontracted rings in a concentration-dependent manner. The maximum relaxation of *E. indica* for the endothelium-intact arteries and the endothelium-denuded arteries were 104.21 ± 3.99% and 107.72 ± 0.58% on aortic rings precontracted with NE and 95.79 ± 3.43% and 101.38 ± 0.80% on those precontracted with KCl, respectively. There were no significant differences between effects on intact and denuded aortic rings. Incubation of intact aortic rings with indomethacin (10^{-4} M) significantly shifted to the right the concentration-effect curves of *E. indica* (Fig. 1). Pretreatment of aortic rings with L-NAME (10^{-4} M) for 30 min. inhibited significantly the relaxation induced by *E. indica* extract only at the concentrations of 100 and 200 µg/mL. The IC_{50} values of the relaxant effect of *E. indica* for 95 ± 3 µg/mL increased to 198 ± 5 µg/mL and 194 ± 6 µg/mL, respectively, in the presence of indomethacin and L-NAME.

DISCUSSION

The present results indicate that *E. indica* induces concentration-dependent relaxation in rat aortic rings. The IC_{50} values of *E. indica* for relaxing contraction of rat thoracic arteries with or without functional endothelium induced by NE or KCl are similar. It suggests

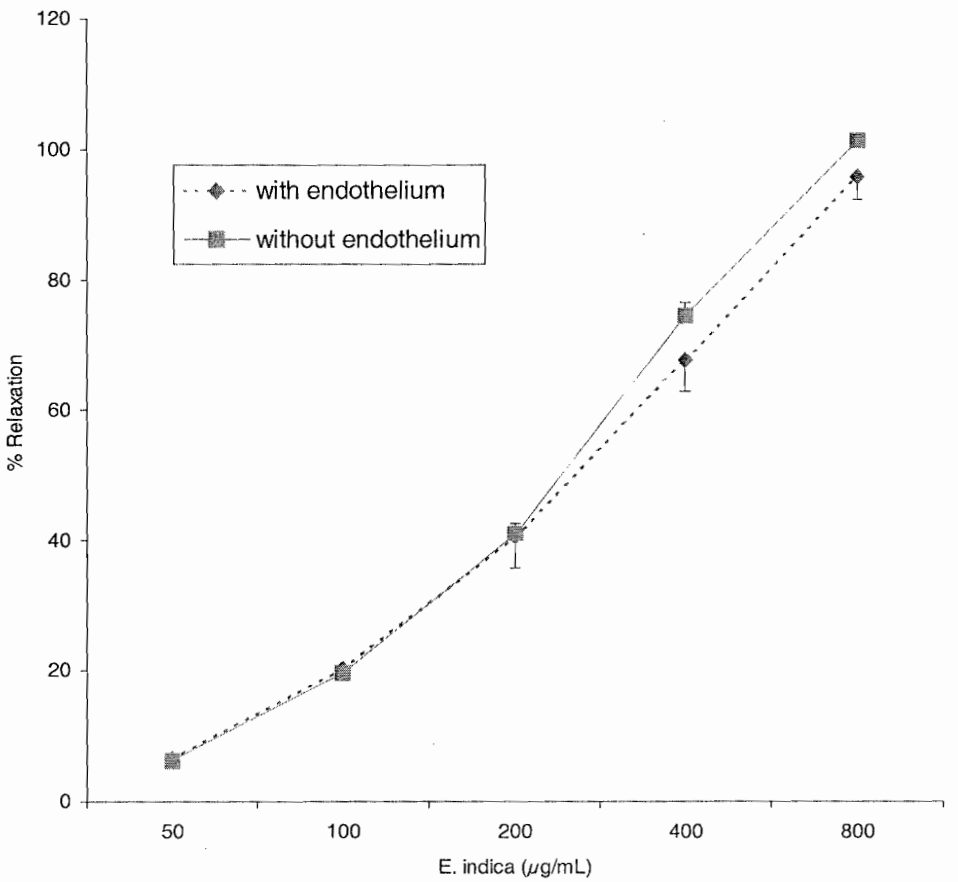

Fig. 2 : Effects of *E. indica* on contractions induced by KCl (60 mM) in endothelium-intact and-denuded aorta. Data are presented as the mean ± SEM. (n = 4).

that the vasorelaxant effects of *E. indica* are independent of endothelium. The vascular endothelium played an important role in controlling vascular tone via the secretion of both relaxant and contractile factors (Hsu et Lin-Shiau, 1995, Kelm et al., 1995, Mizuno et al., 1998). Endothelium cells respond to a variety of neurochemical and physical stimuli by releasing endothelium-dependent vasodilators such as endothelium-derived relaxing factor and prostacyclin (Furchgott et Zawadzki, 1980, Baisch et al., 1998, Calderone at al., 1999). The relaxant action of *E. indica* was significantly reduced in endothelium intact aorta in the presence of indomethacin (a cycloxygenase inhibitor, Corvol, 1993). Thus, the vasorelaxation caused by *E. indica* was mediated at least in part by prostacyclin. L-NAME (a nitric oxide synthethase inhibitor, Dellipizzi et al., 1997), had no effect or only a slight effect on *E. indica*-induced relaxation, thereby excluding the major contribution of nitric oxide to the relaxation of rat aortic rings « *in vitro* ». The concentration of L-NAME used in our aortic ring experiments (10^{-4} M) is more than sufficient to fully inhibit NO synthetase activity. This was verified in the studies by testing the response to acetylcholine, which was completely inhibited in the presence of L-NAME (data not shown). NO acts on the vascular smooth cells, througth the stimulation of the soluble enzyme guanylate cyclase and elevation of the cytosolic cGMP (Calderone et al., 1999).

On the other hand, previous studies revealed the presence of several flavonoids in *E. indica* (Tanaka et al., 2000 ; Azebaze et al., 2000) with antioxidant and free radical scavenging properties. It has also been demonstrated that *E. indica* induces a reduction of blood pressure of cats which was completely blocked by antihistaminic agent, dipheuhydramine (Chatterjee et al., 1981). Our results show that *E. indica* exerts a direct vasodilatory effect on the vasculature, which can explain, at least in part, its hypotensive effect. The relaxation of smooth muscle in the vascular wall in turn is believed to lead to decrease in arterial blood pressure because of a reduction in vascular tone.

The present results indicate that the endothelium-independent relaxation induced by *E. indica* in rat isolated aortic rings may be partially mediated by the activation of endothelial cyclooxygenase which is sensitive to inhibition by indomethacin.

REFERENCES

ADJANOHOUN, J.E., ABOUBAKAR, N., DRAMANE, K ., ZBOT, M.E., EKPERE, J.A., ENOW-OROCK, E.G., FOCHO, D., GBILE, Z.O., KAMANYI, A., KAMSU-KOM, J., KEITA, A., MBENKUM, T., MBI, C.N., MBIELE, A.L., MBOME, L.L., MUBIRU, N.K., NANCY, W.L., NKONGMENECK, B., SABATIE, B., SOFOWORA, A., TAMZE, V., WIRMUM, C .K. (1996) Traditional Medicine and Pharmacopoeia : contibution to ethnobotanical and floristical studies in Cameroon. Organisation of African Unity Scientific, Technical and Research Commission. Centre National de Production de Manuels Scolaires, Porto-Novo.

AYENSU, E.S. (1978). Medicinal Plants of West Africa. PIB. Reference Publication, Algonac, MI, p. 153.

AZEBAZE, G.A., WAFFO, A.K., NKENFACK, A.F., FOMUM, Z.T., MEYER, M., BODO, B., VAN HEERDEN, F.R.(2000). Indicanin A, a new phenylcoumarin from root bark *of Erythrina indica.* Journal of Natural Product 63, 855 – 856.

BAISCH, M.A.L., JOHNSTON, K.B., PAGANINI, S.F.L. (1998). Endothelium-dependent vasorelaxing activity of aqueous extracts of *Ilex paraguariensis* on mesenteric arterial bed of rats. Journal of Ethnopharmacology, 60, 133 – 139

BONNET, A.P., MICHEL, A., LAURENT, F., SABLAYROLLES, C., RECHENCQ, E., MANI, C.I., BOUCARD, M., CAPAT, J.P. (1992). Synthesis and antibronchospastic activities of 8-alkoxy-and 8-(alkylamino) imidazo [1,2-a] pyrazines. Journal of Medicinal Chemistry 325, 90 – 95.

CALDERONE, V., MARTINOTTI, E., BARAGATTI, B., BRESCHI, M.C., MORELLI, I., 1999. Vascular effects of aqueous crude extracts of *Artemisia verlotorum* Lamotte (Compositae) : *in vivo* and *in vitro* pharmacological studies in rats. Phytotherapy Research 13, 645 – 648.

CHATTERJEE, G.K., GURMAN, T.K., NAG CHAUDHURY, A .K., PAL, S.P., 1981. Preliminary pharmacological screening of *Erythrina variegata Var. Orientalis* (Syn. *E. indica*) seeds. Indian Journal of Pharmacology 11 (2),, 153 – 158.

CHAWLA, A.S., KRISHNAN, T.R., JACKSON, A.H., SCALABRIN, D.A., 1998. Alkaloidal constituents of *Erythrina variegata* bark. Planta Medica 54 (6), 526 – 528.

CORVOL, P., 1993. L'endothélium, plaque tournante de la vasomotricité et de la trophicité de la paroi artériell. Médecine/Sciences 9, 1031 – 1033.

COX, P. A., 1993. Saving the ethnopharmacological heritage of Samoa. Journal of Ethnopharmacology 38 (2 – 3), 181 – 188.

DILLIPIZZI, A., PUCCI, L. M., MOSNY, Y. A., DESEYN, K., AND NASJLETTI, A. (1997). Contribution of constrictor prostanoids to the calcium-dependent basal tone in the aorta from rats with aortic coarctation-induced hypertension : relationship to nitric oxide. Journal of Pharmacology and Experimental Therapeutics 283, (1), 75 - 81.

DIMO, T., TAN, P.V., DONGO, E., KAMTCHOUING, P.,RAKOTONIRINA, S.V. (2002). In vitro vascular smooth muscle contractile activity of *Aspilia africana* extract on rat aortic preparations. Pharmazie 57 (6), 421 – 423).

DYKE, S.F., QUESSY, D., 1981. The alkaloids chemistry and physiology, Vol. 18. In : Rodrigo, R.G.A. (Ed.), Academic Press, New Pork, p. 1.

FURCHGOTT, R.F., ZAWADZKI, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373 – 376.

HEGDE, N., 1993. Cultivation and uses of *Erythrina variegata* in Western India. In S.B. Westley and M.H. Powell, eds. *Erythrina in the New and Old Worlds*. Paia, HI (USA) : NFRA, pp. 77 – 84.

HSU, K.S., LIN-SHIAU, S.Y., 1995. Relaxation of rat thoracic aorta induced by pyridine. European Journal of Pharmacology. Environmental Toxicology and Pharmacology Section 292, 265 – 270.

KAZDA, S., HIRTH, C., STASCH, J.P. (1988) Diurectic effect of nitrendipine contributes to its antihypertensive efficacy. A review. Journal of Cardiovascular Pharmacology 12 (4), S1 – S5.

Kelm, M., Feelisch, M., Krebber, T., Deuben, A., Motz, W., Strauer, E. B. (1995). Role of nitric oxide in the regulation of coronary vascular tone in hearts from hypertensive rats. Hypertension 25, 186 - 193.

KIMURA, Y., FUKUI, H., TANAKA ; M., OKAMOTO, M., MORINO, A., MIURA, A., KIMURA, K.,ENOMOTO, H (1986). Antihypertensive effects of a new dihydropyridine calcium antagonism. Arzneimittel-Forschund/Drug Research 36, 1329 – 1335.

MIZUNO, O., HIRANO, K., NISHIMURA, J., KUBO, C., KANAIDE, H. (1998). Mechanism of endothelium-dependent relaxation induced by thrombin in the pig coronary artery. European Journal of Pharmacology 351, 67 - 77.

NKENGFACK, A.E., AZEBAZE, A.G.B., WAFFO, A.K., FOMUM, Z.T., MEYER, M., VAN HEERDEN, F.R., 2001. Cytotoxic isoflavones from *Erythrina indica*. Phytochemistry 58, 1113 – 1120.

SOFOWORA, A. (1983). Medicinal plants and traditional medicine in Africa. John Wiley & Sons Ltd, London, p 1.

TANAKA, H., ETOH, H., SHIMIZU, H., MAKITA, T., TATEISHI, Y., 2000. Two new isoflavonoids from *Erythrina variegata*. Planta Medica 66 –6), 578 – 579.

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