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Original Article

Bioactivity Guided Fractionation of Ethanol Extract of *Caesalpinia digyna* Rottler Roots

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ABSTRACT

Background: Authors have reported earlier that ethanol extract of *Caesalpinia digyna* Rottler roots exhibits significant antianxiety activity at 400 mg/kg, po, in mice using elevated plus-maze (EPM). **Objective:** Aim of the study was to isolate antianxiety principle(s) from ethanol extract of *C. digyna* roots following bioactivity guided fractionation approach. **Materials and Methods:** Bioactive ethanol extract was partitioned with ethyl acetate to get ethyl acetate soluble (EASF) and ethyl acetate insoluble (EAIF) fractions. A compound (CD_1) precipitated from EASF. The two fractions and CD_1 were evaluated for antianxiety activity in mice. Column chromatography of EASF yielded 5 fractions (F_1-F_5), all of which were evaluated for antianxiety activity using EPM. **Results:** Present study revealed that EASF (80 mg/kg) and CD_1 (40 mg/kg) exhibited significant antianxiety activity, while EAIF does not. Among the five fractions, only F_4 (40 mg/kg, po), exhibited significant antianxiety activity, which was statistically comparable to that of diazepam (2 mg/kg). **Conclusion:** Present investigation reveals that EASF obtained by partitioning of ethanol extract of *C. digyna* roots with ethyl acetate, and a compound CD_1, isolated from EASF, exhibit significant antianxiety activity. Among 5 fractions (F_1-F_5) obtained from column chromatography of EASF, only F_4 exhibited significant antianxiety activity. F_4 is being processed further to isolate the anxiolytic constituent(s), and CD_1 is being characterized.

Key words: Antianxiety, Bioactivity-guided fractionation, *Caesalpinia digyna*, Elevated plus-maze.

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INTRODUCTION

Caesalpinia digyna Rottler (*Caesalpiniaceae*) is a large, perennial, prickly shrub or climber. It is commonly known as Teri Pods (English), Vakerimul (Hindi) or Udakiryaka (Sanskrit). Roots of this plant have been used traditionally as astringent, febrifuge and nervine tonic. The roots have marked astringent and antipyretic properties. In some parts of Burma, root powder mixed with water, is drunk as a febrifuge and is said to have soothing effects on nerves. *C. digyna* is one of the ingredients of an indigenous drug preparation Geriforte®, which has been used for curing senile pruritis.[1-2] The formulation has also been reported to exhibit antifatigue and anti-stress effect.[3] Methanol root extract of the plant has been reported to have antioxidant, antidiabetic and radioprotective effect.[4-6] Bergenin, isolated from the roots of *C. digyna* has been reported to exhibit Type 2 antidiabetic activity.[7-8] Earlier studies by the authors have reported that ethanol extract of *C. digyna* roots shows significant antianxiety activity at 400 mg/kg, po, in mice using elevated plus-maze.[9] Continuing with the investigation on the ethanol extract of the plant, a fraction and a pure compound with significant antianxiety activity have been isolated. Authors intend to share these findings through this communication.

MATERIALS AND METHODS

Plant material

Dried roots of *C. digyna* were purchased from Manilal Lallubhai & Co., Mumbai, India. Identity of the plant drug was confirmed through the Head, Raw Materials, Herbarium & Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India, vide letter number NISCAIR/RHMD/Consult/2013/2351-131-3, dated 19.12.2013.

Preparation of extracts

C. digyna roots (1 kg) were packed in a Soxhlet apparatus fitted over 5 L round-bottom flask. The drug was successively extracted with petroleum ether, chloroform and ethanol (3 L each) for 15 h over boiling water bath. Solvents were recovered using rotary vacuum evaporator (EYELA N1100).

Animals

Laca mice (20-30 g) of either sex, procured from the Animal House, Panjab University, Chandigarh, were maintained in a 12 h light/dark cycle at a temperature of 25 ± 2°C. The mice were fed standard pellet diet (Ashirwad Industries, Mohali) and water. Food was withdrawn 4 h before the experiment though water was allowed *ad libitum*. Six mice each were allocated to different experimental groups. All the studies were performed as per the guidelines of the Institutional Ethical Committee of Panjab University, Chandigarh (Approval No. IAEC/411, dated 11. 09. 2013).

Preparation of dose

Carboxy methyl cellulose (0.5% w/v aqueous) containing 5% tween 80 was used as vehicle for preparing the suspension of test samples and diazepam (standard anxiolytic). The vehicle alone served as control. Test material and diazepam (Jawa Pharmaceuticals Pvt. Ltd., Gurgaon) were suspended in the vehicle in such concentrations as to administer appropriate doses of these to mice in a volume ranging from 0.20 to 0.30 ml, po, using a tuberculin syringe fitted with an oral canula.

Bioactivity-guided fractionation

Dried ethanol extract (105 g) was suspended uniformly in 150 ml water, placed in three-necked round bottom flask fitted with Teflon stirrer, and partitioned with ethyl acetate (100 ml) by heating (50°C) for 30 min

Table 1: Antianxiety activity profile of fractions of ethanol extract of C. *digyna roots* and CD_1 using EPM

Treatment	Dose (mg/kg)	mean* number of entries in open arm	mean* time spent in open arm (sec)
Control (Vehicle)	—	2.2 ± 0.30[b]	2.7 ± 0.39[b]
Diazepam	2	7.2 ± 0.60[a]	14.2 ± 1.14[a]
EASF	80	3.3 ± 0.76[b]	7.4 ± 0.52[a,b]
EAIF	275	2.3 ± 0.33[b]	2.9 ± 0.67[b]
CD_1	40	5.5 ± 0.76[a]	8.3 ± 0.92[a,b]

*n=6, mean ± SEM. [a]p<0.05 vs. control; [b]p<0.05 vs. diazepam; one way ANOVA followed by Tukey's multiple range test.

Table 2: Antiaxiety activity profile of fractions, obtained after column chromatography of EASF, using EPM

Treatment	Dose (mg/kg)	Mean* number of entries in open arm	Mean* time spent in open arm (sec)
Control (Vehicle)	—	2.2 ± 0.31[b]	2.7 ± 0.39[b]
Diazepam	2	7.2 ± 0.60[a]	14.2 ± 1.14[a]
F_1	2	2.2 ± 0.31[b]	3.2 ± 0.58[b]
F_2	3	2.0 ± 0.36[b]	2.8 ± 0.68[b]
F_3	4	2.7±0.33[b]	3.1±0.58[b]
F_4	40	4.5 ± 0.62[a,b]	6.2 ± 0.81[a,b]
F_5	30	2.2 ± 0.31[b]	2.9 ± 0.65[b]

*n=6, mean ± SEM. [a]p<0.05 vs. control; [b]p<0.05 vs. diazepam; one way ANOVA followed by Tukey's multiple range test.

with continuous stirring. The procedure was repeated five times. All the ethyl acetate fractions were pooled. Solvents from the pooled fractions and the remaining aqueous fraction were recovered under reduced pressure using rotary vacuum evaporator to get ethyl acetate soluble fraction (EASF) and ethyl acetate insoluble fraction (EAIF) respectively.

A solid brown material precipitated from the concentrated EASF, was separated, washed (×3) with ethanol and recrystallized using methanol to get a crystalline compound CD_1. Antianxiety activity of EASF, EAIF and CD_1 was evaluated at 80, 275 and 40 mg/kg, po, respectively.

EASF (33 g) was subjected to column chromatography using silica gel (#60-120, s.d. fine-Chem Ltd, Mumbai; 900 g). Elution was done with pet ether, chloroform and methanol, in appropriate proportions. A total of 158 fractions, each of 500 ml, were collected and pooled on the basis of TLC profile to get five fractions - F_1-F_5. These were subjected to antianxiety activity evaluation at 2, 3, 4, 40 and 30 mg/kg, po, respectively.

Antianxiety activity

Antianxiety activity was evaluated using the modified elevated plus-maze (EPM).[10-11] The apparatus comprising two open arms (16×5 cm) and two closed arms (16×5×12 cm) having an open roof, was kept elevated (25 cm) from the floor for evaluating antianxiety behavior in animals. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze, could invoke anxiety in mice. Doses were administered orally using tuberculin syringe fitted with an oral canula. The dose administration schedule was so adjusted that each mouse was having its turn on the EPM 60 min after the administration of vehicle, diazepam or the test material. Each mouse was placed at the center of EPM with its head facing towards the open arm. During 5 min duration of the experiment, behavior of the mouse was recorded as (a) the number of entries into the open arms and (b) mean time spent by the mouse in open arms.

Statistical analysis

The data have been expressed as mean ± standard error of mean (SEM). Significant differences among the groups were assessed using one way analysis of variance (ANOVA) using GraphPad Prism 5. The test was followed by Tukey's multiple range test; p values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Previous studies by the authors have reported that among the four extracts namely, petroleum ether, chloroform, ethanol and water of *C. digyna* roots, only ethanol extract exhibits significant antianxiety activity in mice at 400 mg/kg, po., using EPM.[9] In the present investigations, doses of the test materials were selected taking into account their yield. It was planned to subject bioactive ethanol extract of the plant to bioactivity guided fractionation with a view to isolate antianxiety fraction(s) or compound(s). Thus, the ethanol extract was partitioned with ethyl acetate to get EASF (19.8% w/w), EAIF (69.0% w/w) and CD_1 (10.8% w/w). Results of antianxiety activity evaluation of EASF, EAIF and CD_1 are shown in Table 1. EASF and CD_1 exhibited significant antianxiety activity at a dose of 80 and 40 mg/kg, po, respectively. However, EAIF did not show any antianxiety effect on mice.

EASF was further subjected to column chromatography. A total of 158 fractions, each of 500 ml, were collected during column chromatography of EASF. These were pooled on the basis of similar TLC patterns to get five fractions - F_1 (1.7%), F_2 (4.1%), F_3 (4.9%), F_4 (49.0%) and F_5 (36.1%). All the five fractions were evaluated for antianxiety activity at 2, 3, 4, 40 and 30 mg/kg, po (Table 2). Fraction F_4 exhibited significant antianxiety activity at 40 mg/kg, po, using EPM. F_4, eluted using chloroform-methanol (98:2), showed one major and five minor spots on TLC (mobile phase: toluene-ethyl acetate, 7.5:2.5). F_4 shall be subjected to column chromatography, and CD_1 is in the process of being characterized.

CONCLUSION

Present investigation reveals that ethyl acetate soluble fraction obtained by partitioning of ethanol extract of *Caesalpinia digyna* roots with ethyl acetate, and a compound CD_1, isolated from ethyl acetate soluble fraction, exhibit significant antianxiety activity. Out of five fractions

(F_1-F_5) obtained by column chromatography of EASF, only F_4 exhibits significant antianxiety activity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATION USED

EASF: Ethyl acetate soluble fraction; **EAIF:** Ethyl acetate insoluble fraction; **EPM:** Elevated plus-maze.

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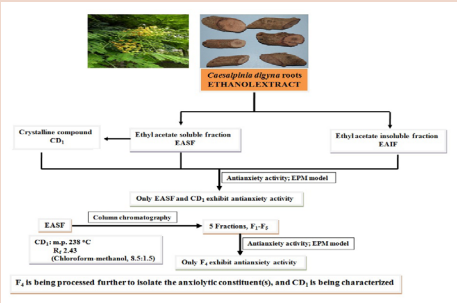
PICTORIAL ABSTRACT



SUMMARY

- Bioactive ethanol extract of *Caesalpinia digyna* Rottler roots was subjected to antianxiety activity guided fractionation.
- A crystalline compound (CD_1) precipitated from ethyl acetate soluble fraction (EASF) obtained from the ethanol extract.
- A fraction F_4 obtained from column chromatography of EASF, and CD_1 exhibit significant antianxiety activity in mice at 40 mg/kg, po, which is statistically comparable to that of diazepam (2 mg/kg, po)
- F_4 is being processed further to isolate the anxiolytic constituent(s), and CD_1 is being characterized.

ABOUT AUTHORS

Anupam Sharma: Is Professor of Pharmacognosy at University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh. Professor Sharma has more than 30 years of teaching and research experience. He has guided 10 Ph.D. and 17 M Pharm students. He has 74 national and international publications. His major research areas include "Investigating plant drugs with a view to isolate bioactive constituents; characterization of phytoconstituents; developing standardization parameters for plant drugs".

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