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COMPARATIVE ANTIANXIETY ACTIVITY EVALUATION OF *ARGYREIA SPECIOSA* LINN. (ROOTS), *CAESALPINIA DIGYNA* ROTTLER (ROOTS) AND *SPHAERANTHUS INDICUS* LINN. (FLOWERS)

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ABSTRACT: Aim of the present study was a comparative evaluation of antianxiety activity of three traditionally used medicinal plants — *Argyreia speciosa* (Roots), *Caesalpinia digyna* (Roots) and *Sphaeranthus indicus* (Flowers). The activity of various extracts (petroleum ether, chloroform, ethanol and water) of all the three plants was evaluated using elevated plus-maze model. The studies were conducted on lacca mice, and the test materials were administered per oral route. Results indicate that the ethanol extract of *C. digyna* roots exhibited maximum and significant dose dependent effect at 200 and 400 mg/kg on elevated plus-maze, the results were comparable to the standard antianxiety drug diazepam (2 mg/kg).

INTRODUCTION: *Argyreia speciosa* (Linn.f.) Sweet, [syn. *A. nervosa* (Burm. f.) Bojer] of Convolvulaceae family is a perennial climbing shrub with woody tomentose stem. It is commonly known as Hawaiin Baby Woodrose, Samudra-sok or Vriddhadaruka. It is an important rasayana drug in Ayurveda. The plant is traditionally used for therapy of diseases like nervousness, bronchitis, tuberculosis, arthritis, diabetes, in stomach complaints, sores on foot, small pox, syphilis, dysentery and diarrhea. In Hindoo medicine, the roots of *A. speciosa* is regarded as alternative tonic and useful in rheumatic affections and diseases of the nervous system[1-5]. *Caesalpinia digyna* Rotl. (Caesalpiniaceae) is a large, perennial, prickly shrub or climber. It is commonly known as Teri Pods, Vakerimul or Udakiryaka.

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Roots of the plant 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 [2, 5].

Sphaeranthus indicus Linn. (Asteraceae) is an annual herb, commonly known as East Indian globe thistle, Gorakhmundi or Mahamundi. It is widely used in Ayurveda to treat epilepsy, mental tension, diabetes, jaundice, leprosy, constipation, helminths and epilepsy. The oil prepared using the plant roots is reportedly useful in treating scrofula, and as an aphrodisiac. External application of a paste of *S. indicus* is beneficial in treating pruritis, edema, arthritis, filariasis, gout and cervical adenopathy [4-6]. Petroleum ether extract of flowers of the plant has been reported to possess antianxiety activity [7].

Plants have been used traditionally for their ameliorative effect in anxiety since the ancient times. However, their phytochemical and

pharmacological data are scanty and controversial [8]. Literature survey has revealed that despite a long history of use of *A. speciosa*, *C. digyna* and *S. indicus* as traditional medicines for the treatment of various ailments, including nervousness and mental tension, these plants have not been investigated scientifically for substantiating their therapeutic claims.

So, based on their traditional use, it was proposed to investigate *A. speciosa*, *C. digyna* and *S. indicus* for antianxiety activity. Thus, the objective of the present study was to evaluate and compare the antianxiety activity of various extracts viz. petroleum ether, chloroform, ethanol and water extracts of *A. speciosa*, *C. digyna* and *S. indicus*.

MATERIALS AND METHODS:

Procurement and authentication of plant material: Dried roots of *A. speciosa*, *C. digyna*, and dried flowers of *S. indicus* were purchased from Manilal Lallubhai & Co., Mumbai, India. Identity of the plant drugs was confirmed through Head, Raw Materials, Herbarium & Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India, vide letter dated19.12.2013 bearing numbers NISCAIR/RHMD/Consult/2013/2351-131-1 (*A. speciosa* roots); -131-2 (*S. indicus* flowers) and -131-3 (*C. digyna*, roots).

Chemicals: Petroleum ether 60-80°C (Merck India Ltd., Mumbai), chloroform (Thermo Fisher Scientific India Pvt. Ltd., Mumbai), ethanol (Panipat Sugar Mill, D-Unit, Panipat), carboxy methyl cellulose (Merck Specialities Pvt. Ltd., Mumbai), tween 80 (HiMedia Laboratories Pvt. Ltd., Mumbai) and diazepam (Jawa Pharmaceuticals Pvt. Ltd., Gurgaon) were used in the present investigation.

Preparation of extracts:

Coarsely powdered *A. speciosa* and *C. digyna* roots, and *S. indicus* flowers (200 g each) were subjected to exhaustive soxhlet extraction using petroleum ether, chloroform, ethanol and distilled water. Solvents were recovered from all extracts using Eyela N 1100 rotary vacuum evaporator under reduced pressure, and the extracts were preserved in vacuum desiccator containing anhydrous silica gel blue.

Experimental animals:

Lacca 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 constant temperature of 25°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*. The animals were allocated to different experimental groups each of six mice. All the studies were performed as per the guidelines of the Institutional Ethical Committee of Panjab University, Chandigarh, CPCSEA, India (Approval No. IAEC/411, dated 11.09.2013).

Vehicle and standard:

Carboxy methyl cellulose (0.5% w/v aqueous) containing 5% tween 80 was used as vehicle for preparing the suspension of various test doses of different extracts. Diazepam, suspended in the vehicle, was used as the standard antianxiety drug. The vehicle alone served as control.

Preparation of doses:

Test material were suspended in the vehicle in such concentrations as to administer 200 or 400 mg/kg extracts and 2 mg/kg of diazepam to mice in a volume ranging from 0.20 to 0.30 ml, per oral route, using a tuberculin syringe fitted with an oral canula.

Pharmacological Evaluations:

Acute oral toxicity studies: Acute toxicity studies for all the extracts were conducted as per OECD guidelines 423 [9].

Elevated plus-maze model of anxiety: Antianxiety activity was evaluated using the modified elevated plus-maze (EPM) [10, 11]. The apparatus comprising two open arms (16x5 cm) and two closed arms (16x5x12 cm) having an open roof, with the plus-maze elevated (25 cm) from the floor was used to evaluate 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 elevated plus-maze apparatus 90 minutes after the administration of the test extract, diazepam or vehicle. Each mouse was placed at the center of EPM with its head facing towards the open arms. During the 5 min duration of the experiment, behavior of the mouse was recorded as (a) the number of entries into the open arms, (b) mean time spent by the mouse in each of the 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). The test was followed by Tukey's multiple range test, p values less than 0.05 were considered as significant.

Phytochemical Screening: The extract exhibiting maximum antianxiety activity was subjected to phytochemical screening in order to identify the major class of constituents present in the extract [12].

RESULTS:

Percentage yield of extracts: Percentage yield of extracts is listed in **Table 1**.

Oral acute toxicity studies: All the extracts of *A. speciosa*, *C. digyna* and *S. indicus* neither exhibited signs of acute toxicity nor mortality upto the dose of 2000 mg/kg, p.o.

Antianxiety activity:

Table 2 summarizes results of the antianxiety activity studies of various extracts.

Phytochemical screening: Ethanol extract of *C. digyna* roots revealed the presence of flavonoids, phytosterols, terpenoids, tannins, phenols, amino acids and carbohydrates.

DISCUSSION: The elevated plus-maze is a well-established and most widely used animal model in contemporary preclinical research on anxiety. Fear due to height induces anxiety in the animals when placed on the EPM [10, 11]. Antianxiety effects of all the test extracts were compared with the standard antianxiety drug diazepam. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer place (closed arm). Antianxiety agents are expected to increase the motor activity, which is measured by number of entries and time spent by the animal in the open arm [10]. Results of the present study indicate that the ethanol extract of *A. speciosa*, *C. digyna* and chloroform extract of *S. indicus* showed significant dose dependant antianxiety activity at 200 mg/kg and 400 mg/kg (**Table 2**).

TABLE 1: PERCENTAGE YIELD (w/w) OF VARIOUS EXTRACTS OF ROOTS OF *A. SPECIOSA* AND *C. DIGYNA*, AND *S. INDICUS* FLOWERS.

Extract	*A. speciosa*	*C. digyna*	*S. indicus*
Pet ether	1.33 (ASPE)	1.10 (CDPE)	5.18 (SIPE)
Chloroform	1.95 (ASCE)	2.62 (CDCE)	2.26 (SICE)
Ethanol	5.87 (ASEE)	10.56 (CDEE)	6.96 (SIEE)
Distilled Water	10.31 (ASWE)	14.30 (CDWE)	10.88 (SIWE)

TABLE 2: ANTIANXIETY ACTIVITY PROFILE OF VARIOUS EXTRACTS OF ROOTS OF *A. SPECIOSA* AND *C. DIGYNA*, AND *S. INDICUS* FLOWERS 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 mg/kg	7.2±0.60[a]	14.2±1.14[a]
	200 mg/kg	2.2±0.31[b]	2.8±0.45[b]
ASPE	400 mg/kg	2.3±0.31[b]	2.7±0.39[b]
	200 mg/kg	2.2±0.48[b]	2.8±0.33[b]
ASCE	400 mg/kg	2.3±0.33[b]	3.2±0.31[b]
ASEE	200 mg/kg	4.8±0.14[a, b]	8.8±0.19
	400 mg/kg	5.7±0.23[a]	9.1±0.04[a]
	200 mg/kg	2.8±0.31[b]	3.9±0.52[b]

ASWE	400 mg/kg	3.2±0.60[b]	5.3±0.59[b]
CDPE	200 mg/kg	2.2±0.33[b]	3.1±0.23[b]
	400 mg/kg	2.3±0.15[b]	2.5±0.26[b]
	200 mg/kg	3.8±0.19 [b]	3.6±0.52 [b]
CDCE	400 mg/kg	4.3±0.49[a, b]	5.1±0.35 [b]
	200 mg/kg	5.7±0.13[a]	9.2±0.17[a]
CDEE	400 mg/kg	6.5±0.21[a]	13.8±1.05[a]
	200 mg/kg	2.3±0.42[b]	3.1±0.33[b]
CDWE	400 mg/kg	2.2±0.40[b]	3.6±0.25[b]
	200 mg/kg	3.9±0.36 [b]	6.4±0.29 [b]
SIPE	400 mg/kg	4.8±0.17[a, b]	8.9±0.52
	200 mg/kg	3.7±0.56[b]	7.9±0.48 [b]
SICE	400 mg/kg	4.5±0.76[a,b]	9.5±0.63[a]
	200 mg/kg	2.3±0.21[b]	2.8±0.14[b]
SIEE	400 mg/kg	2.3±0.36[b]	3.3±0.49[b]
SIWE	200 mg/kg	2.2±0.31[b]	2.3±0.22[b]
	400 mg/kg	2.5±0.43[b]	2.6±0.28[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.

However, among all the extracts, ethanol extract of *C. digyna* roots exhibited maximum antianxiety activity at 400 mg/kg dose, and the activity was statistically comparable to diazepam. Preliminary phytochemical investigation of ethanol extract of *C. digyna* roots revealed that it contains a variety of phytoconstituent groups including flavonoids, terpenoids, tannins and phenols. The present study confirms the earlier reported [7] antianxiety activity of pet ether extract of *S. indicus* flowers.

CONCLUSION: Present investigation reveals that out of 12 extracts of the three investigated plants, ethanol extract of *C. digyna* roots exhibits maximum and significant antianxiety activity which is statistically comparable to the diazepam. Further studies are being undertaken to isolate the active constituent(s) responsible for antianxiety effect of *C. digyna* roots.

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