ORIGINAL ARTICLE

Pharmacognostic and pharmacological screening of *Ageratum conyzoides* stem extract for its antianxiety potential

Saurabh Satija[1], Saloni Kamboj[2], Jaspreet Kaur[1], Ripdaman[3], Manish Vyas[1], Meenu Mehta[1]

[1]Department of Pharmaceutical Sciences, Lovely Professional University, Phagwara, Punjab, India, [2]Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab, India, [3]Health Care Aid, St. Bonafice Hospital, 409 Tache Avenue, Winnipeg, Canada

Abstract

Objective: The current study assessed the antianxiety potential of standardized stem extracts of *Ageratum conyzoides*. **Materials and Methods:** Methanol extract of *A. conyzoides* stem was prepared using Soxhlet apparatus. Phytochemical investigation was done using standard procedures. Fractionation of methanol extract was done using liquid-liquid extraction. Methanol extract and its fractions (ethyl acetate and butanol) were evaluated for antianxiety potential using elevated plus maze model. **Results and Discussion:** The methanol extract gave the yield of 12% w/w and its ethyl acetate and butanol fractions gave 5.03% w/w and 2.0% w/w, respectively. The methanol extract of *A. conyzoides* showed a significant increase in mean of a number of entries by mice in open arms and average time spent when compared to control group. Results revealed that ethyl acetate fraction was responsible for its anxiolytic effect. **Conclusion:** The present study validates the traditional claim of *A. conyzoides* as anxiolytic drug as methanol extract of stem produced a significant antianxiety effect.

Key words: *Ageratum conyzoides*, anxiety, extraction, methanol extract, standardization

INTRODUCTION

Herbal medications have been utilized since antiquated times as pharmaceutical for the treatment of a scope of illnesses. Medicinal plants have assumed a key part in well-being of individuals. Notwithstanding the immense advances saw in current pharmaceutical in late decades, plants still influence an imperative commitment to health care.[1,2] *Ageratum conyzoides* is an erect, herbaceous yearly, 30–80 cm tall, stems are secured with fine white hairs, and leaves are inverse, pubescent with long petioles and incorporate glandular trichomes. It is a tropical plant local from Tropical America. It was presented as a decorative plant in 1860. Later, it got away as weed in different environments all through India.[3] Herb contains friedelin, sterol, ß-sitosterol, stigmasterol and α-spinasterol, hydrocarbons, coumarin, flavonoids, hydrocarbons, caryophyllene, quercetin, kaempferol, chromone, phenol, essential oil\ and anti-gonadotropic hormones, and precocenes 1 and 2.[4,5] Leaves and stem contain alkaloids, stigmasterol (major), dotriacontane, fumaric, caffeic acids, 7-OMe-2, 2-di-Me-chromen (e), and new flavone, 5'-OMe-nobiletin. Leaves also contain stigmat-7-en-3-ol (major component).[6,7]

A. conyzoides had been customarily utilized as a part of the treatment of skin illnesses and as wound recuperating agent. A decoction of the plant is taken orally to treat diarrhea and to relieve pain related with navel in children.[5] In Central Africa, the plant is utilized to treat, especially, wounds caused by burns. In Brazil folk prescription, restorative teas of *A. conyzoides* are utilized as anti-inflammatory, pain relieving, and hostile to diarrheic. In India, leaves are utilized to coagulate blood and stems are utilized as hepatoprotective and against bacterial agent.[8]

Address for correspondence:
Dr. Meenu Mehta, Department of Pharmaceutical Sciences, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara – 144 411, Punjab, India.Tel.: +91-7988924829.
E-mail: meenu18288@gmail.com

Received: 11-06-2018
Revised: 24-06-2018
Accepted: 29-06-2018

MATERIALS AND METHODS

Plant Material

The dried plant of *A. conyzoides* was collected from Sri Venkateswara University, Tirupati, Andhra Pradesh and authenticated by the Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupati. A voucher specimen was prepared and deposited in the herbarium, Guru Nanak Dev University, Amritsar, Punjab, under the collection number 993.

Preparation of Extracts

The powdered stem (250 g) was subjected to progressive Soxhlet extraction for 48 h by utilizing solvents in raising polarity, namely petroleum ether (60–80°C) and methanol. Each concentrate was concentrated by refining off the solvent utilizing rotavapor and after that dissipated to dryness on the water bath. Concentrates were weighed, and percentage yield was computed as far as the air-dried weight of the plant material. Plan of extraction is shown in Figure 1.

Fractionation of the Methanol Extract

About 9 g of the methanol extract was fractionated by dissolving in 50 mL of distilled water. At that point, extraction was completed with hexane (100 ml) and aqueous phase was collected. After that, progressive liquid extraction of aqueous phase was over with ethyl acetate and n-butanol (3 × 130 ml). Ethyl acetate part and butanol part were concentrated utilizing a rotary evaporator.

Phytochemical Screening

All concentrates and fractions were subjected to phytochemical screening to recognize the presence of different phytochemicals, namely alkaloids, flavonoids, tannins, anthocyanins, and saponins.[9,10]

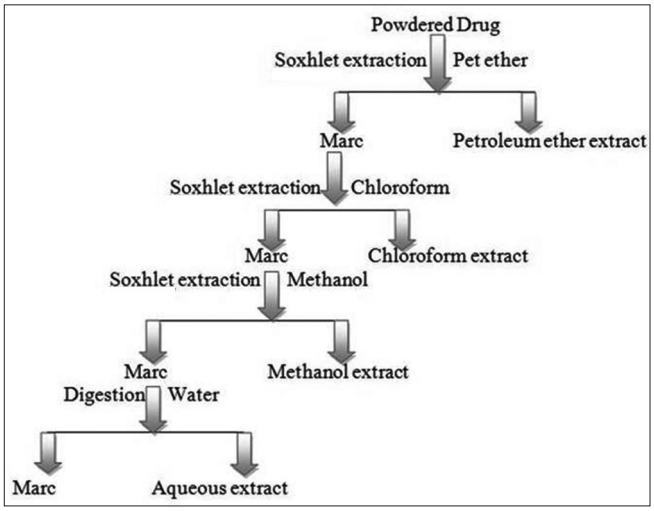

Figure 1: Scheme of extraction

Drugs

Different fractions of the methanolic extract of *Ageratum conyzoides* were used to evaluate anxiolytic activity. Diazepam (DZP) (DZP, 2.0 mg/kg, Sigma, India) was used as the standard anxiolytic drug (positive control group). Carboxymethyl cellulose (CMC) (CMC, 1% w/v, Merck, India) was employed to treat negative control group.

Animals

Swiss albino mice measuring 25–30 g were obtained from the Institute of Integrated Medicine, Jammu. The creatures were kept in the room of controlled conditions of 24 ± 1°C and 12 h light–12 h dark cycles, with free access to food and water.

Experimental Protocol

Six mice in each group were utilized as a part of all sets of experiments. Analyses were completed in a noise-free territory with controlled lighting, between 8:00 a.m. and 12:00 p.m. All conventions and examinations were led in strict compliance as per the ethical standards and guidelines gave by the Institutional Animal Ethical Committee. Different creature groups were utilized in the present investigation as per the following:

- Group I - Vehicle treated: CMC (1% w/v) was administered orally
- Groups II and III - methanol extract (100 and 200 mg/kg)
- Groups IV and V - ethyl acetate fraction of methanol extract (25 and 50 mg/kg)
- Groups VI and VII - butanol fraction of methanol extract (25 and 50 mg/kg)
- Groups VIII - positive control: DZP (2 mg/kg).

The anxiolytic action of methanol concentrate and its fraction (ethyl acetate and butanol) was assessed by utilizing elevated plus maze (EPM). All the test and standard medications were regulated to mice 30 min preceding examination by means of oral route.

EPM

EPM consisted of two open arms (15 cm × 10 cm) and two closed arms (50 cm ×10 cm ×40 cm) stretching out from a platform (5 cm × 5 cm) in the midway and raised to a height of 45 cm above the ground.[11] Every creature was situated at the focal point of the maze confronting one of the encased arms, 30 min after administration of medications. A number of sections and time spent in closed and open arms were recorded for 10 min. Passage into an arm was

Table 1: Preliminary phytochemical screening of extracts

Phytochemical constituents	Hexane extract	Chloroform extract	Methanol extract	Aqueous extract
Carbohydrates				
Molisch's test	–	–	–	+
Fehling's test	–	–	–	+
Benedict's test	–	–	–	+
Proteins				
Biuret test	–	–	–	+
Millon's test	–	–	–	+
Xanthoprotein test	–	–	–	+
Amino acids				
Millon's test	–	–	+	+
Lipids				
Sudan IV test	+	–	–	–
Solubility test	+	–	–	–
Steroids				
Salkowski test	–	–	–	–
Liebermann–Burchard test	–	–	–	–
Alkaloids				
Mayer's test	–	+	+	+
Hager's test	–	+	+	+
Wagner's test	–	+	+	+
Saponins				
Froth formation test	–	+	–	–
Glycosides				
Keller–Killani test	–	–	+	–
Legal test	–	–	+	–
Borntrager's test	–	–	+	–
Modified Borntrager's test	–	–	+	–
Flavonoids				
Shinoda test	–	–	+	–
Lead acetate test	–	–	+	–

+: Present, –: Absent

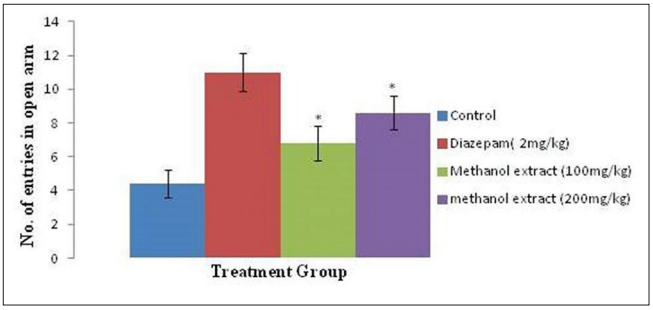

Figure 2: Effect of methanol extract of *Ageratum conyzoides* on a number of entries in open arms by mice

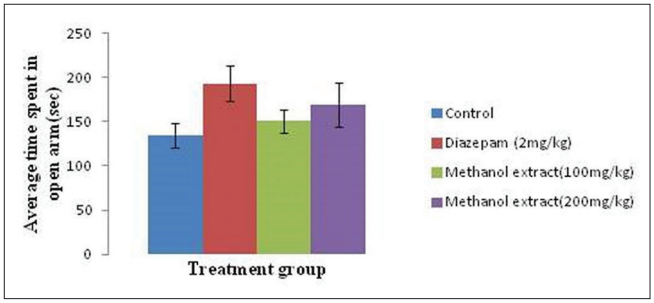

Figure 3: Effect of methanol extract of *Ageratum conyzoides* on average time spent in the open arms by mice

expressed as the creature setting every one of the four paws on the arm. The maze was cleaned appropriately with 5% alcohol, each time before putting the creature, to abolish the conceivable prejudice because of the fragrance left by the former creature.

Statistical Analysis

All data are expressed as mean ± standard deviation and analyzed statistically by one-way analysis of variance followed by Tukey's multiple range test using GraphPad Prism 5.0 software. A $P < 0.05$ was considered statistically significant.

RESULTS

Percentage Yield and Phytochemical Screening of Prepared Extracts and Fractions

The methanol extract gave the yield of 12% w/w and its ethyl acetate and butanol fractions gave 5.03% w/w and 2.0% w/w, respectively. Preliminary phytochemical screening of methanol extract revealed the presence of numerous phytochemical groups [Table 1]. Methanol extract showed the presence of glycosides, alkaloids, and flavonoids.

Evaluation of Anxiolytic Activity of Methanol Extract and its Fractions

The methanol concentrate of *A. conyzoides* demonstrated huge increment in mean of a number of entries by mice in open arms and normal time spent when contrasted with control group [Figures 2 and 3] affirming the conventional claim of plant. In any case, the observed anxiolytic impact at a dosage of 100 mg/kg of methanol extract was not significantly different when contrasted with anxiolytic impact of the same concentrate at measurements of 200 mg/kg. Administration of DZP (2 mg/kg) to mice essentially ($P < 0.05$) expanded the mean time spent by mice in open arms and the average number of entries into the open arms of EPM, affirming an anxiolytic impact.

Among the prepared portions of methanol extract, just ethyl acetate part (25 and 50 mg/kg) demonstrated noteworthy ($P < 0.05$) against anxiety action which was affirmed by an increment in average time spent and mean of a number of entries by mice in open arms of EPM when contrasted with control group. The butanol part of methanol concentrate of *A. conyzoides* did not demonstrate any noteworthy antianxiety activity when contrasted with control [Figures 4 and 5].

DISCUSSION

A. conyzoides is a plant species that is extensively utilized as a solution for mental disorders. In the present investigation, *A. conyzoides* was assessed for anxiolytic impact keeping in mind the end goal to experimentally approve the conventional claim by utilizing behavioral models, namely EPM. The oral administration of methanol extract (100 mg/kg and 200 mg/kg) to mice demonstrated antianxiety impacts showed

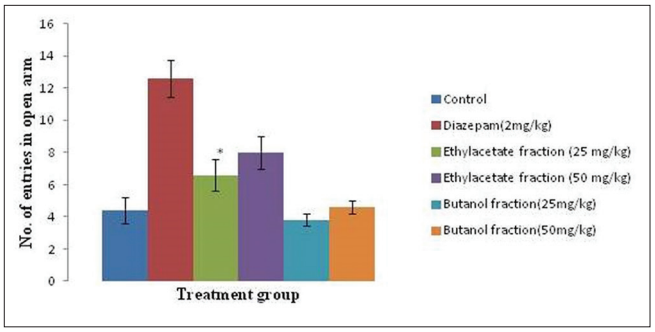

Figure 4: Effect of ethyl acetate and butanol fractions of methanol extract of *Ageratum conyzoides* on a number of entries in open arms by mice

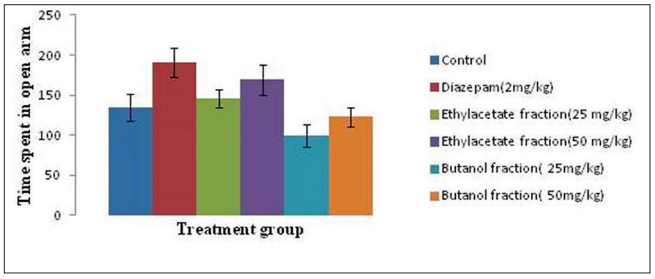

Figure 5: Effect of ethyl acetate and butanol fractions of methanol extract of *Ageratum conyzoides* on average time spent in the open arms by mice

by an increment in average time spent and number of entries in open arm of EPM. Phytochemical screening of methanol extract demonstrated the presence of flavonoids alongside other phytochemical groups. Mechanism of the action of polyphenolic mixes includes the communication with γ-aminobutyric acid (GABAA) receptors at benzodiazepine (BZD) and non-BZD destinations with different affinities to various subunits, serotogenic 5-hydroxytryptamine 1A and 5-hydroxytryptamine 2A/C receptors, noradrenergic and dopaminergic systems, glycine and glutamate receptors, and κ-opioid receptors and cannabinoid receptors.[12] Neuroprotective appearance of flavonoids has been credited to their general bioavailability and *in vivo* occurrence in the brain.[13] Results of this examination showed that the methanolic concentrate of *A. conyzoides* stem had anxiolytic impacts. The phytoconstituent that may in charge of the observed focal impacts was polyphenolic mixes which acts through the various mechanism of action.

CONCLUSION

The present investigation approves the conventional claim of *A. conyzoides* as anxiolytic medication as methanol concentrate of stem produced a significant antianxiety effect. Phytochemical examination distinguished flavonoids as one of the real aggravates that might be in charge of the observed antianxiety effect of the plant. Since there is a need for new more secure and cost-effective anxiolytic compunds having the least side effects when contrasted with manufactured

medicine, *A. conyzoides* can go about as a potential lead for drug development.

REFERENCES

1. Calixto JB, Barz J. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Med Biol Res 2000;33:179-89.
2. Mehta M, Satija S, Kalsi V. *In vitro* Antioxidant evaluation of *Psidium guajava* strem extracts. Int J Drug Dev Res 2011;3:213-6.
3. Dogra KS, Kohli RK. An assessment and impact of three invasive species in the Shivalik Hills of Himachal Pradesh, India. Int J Biodivers Conservation 2009;1:4-10.
4. Dubey S, Gupta KC, Matsumoto T. Sterols of *Ageratum conyzoides* L. Herba Hung 1989;28:71-6.
5. Okaunde AL. Review *Ageratum conyzoides* L. Fitoterpia 2002;73:1-16.
6. Vyas AV, Mulchandani NB. Polyoxygenated flavones from *Ageratum conyzoides.* Photochemistry 1986;25:2625-7.
7. Wiedenfield H, Roder E. Pyrrolizidine alkaloids from *Ageratum conyzoides*. Plant Med 1991;57:578-9.
8. Jain A, Katewa SS, Galav PK, Sharma P. Medicinal plant diversity of Sitamata wildlife sanctuary, Rajasthan, India. J Ethnopharmacol 2005;102:143-57.
9. Cannell RJ. How to approach the isolation of a natural product. Natural Products Isolation (Methods in Biotechnology). New Jersey: Humana Press, Totowa; 1998. p. 343-58.
10. Evans WC, editor. Trease and Evans Pharmacognosy. 15th ed. New Delhi: Elsevier; 2002.
11. Bourin M, Petit-Demouliere B, Dhonnchadha BN, Hascoet M. Animal models of anxiety in mice. Fundam Clin Pharmacol 2007;21:567-74.
12. Herrera-Ruiz M, Roman-Ramos R, Zamilpa A, Tortoriello J, Jimenez-Ferrer JE. Flavonoids from *Tilia americana* with anxiolytic activity in plus-maze test. J Ethnopharmacol 2008;118:312-7.
13. Dajas F, Rivera-Megret F, Blasina F, Arredondo F, Abin-Carriquiry JA, Costa G. Neuroprotection by flavonoids. Braz J Med Biol Res 2003;36:1613-20.

Source of Support: Nil. **Conflict of Interest:** None declared.