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# Screening of antidepressant potential of leaves of Araucaria columaris

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## Abstract

The present work was undertaken with an objective to establish the anti-depressant action of ethanolic leaf extract of *Araucaria columnaris*. The yield of the ethanolic extract was found to be 36.9% and the extract was black in color. The preliminary phytochemical analysis suggests the presence of cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The antidepressant action of the extract (EEAC) was tested using two animal models at two dose levels (200 and 400 mg/kg) using forced swim test (FST) and tail suspension test (TST) models. The immobility time in the TST for EEAC (400 mg/kg) was found to be 18.33 ± 1.994 sec and was significantly comparable ($p$<0.0001) to that of fluoxetine at a dose on 10mg/kg (9.67 ± 0.667 sec). The swimming frequency in the FST increased significantly at both the dose levels of EEAC and it surpassed fluoxetine (43.00 ± 1.211) at 400 mg/kg dose of EEAC (44.00 ± 1.317).

**Keywords:** *Araucaria columnaris,* extract, forced swim test, tail suspension test, antidepressant, fluoxetine

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## Introduction

Depression is a medical condition with a complex biological pattern of aetiology, involving genetic and epigenetic factors, along with different environmental stressors. Recent evidence suggests that oxidative stress processes might play a relevant role in the pathogenic mechanism(s) underlying many major psychiatric disorders, including depression (Scapagnini et al., 2012). This suggests the role of antioxidants in treatment of depression.

Several plant parts, extracts thereof and isolates have been reported in literature to possess pharmacological actions. In a pursuit to obtain natural antidepressant molecules that may not show the potential for abuse, the present investigation was undertaken. *Araucaria columnaris* is an ornamental plant that has been less explored scientifically for its medicinal value. The presence of flavonoids though has been reported in the plant. The plant has been reported to possess antibacterial (Saranya Devi et al., 2014), antioxidant (Zaffar et al., 2014), anticancer (Aslam et al., 2015) and gastroprotective actions (Schmeda-Hirschmann et al., 2005).

The objective of the present investigation was to explore the antidepressant action of the ethanolic extract of *Araucaria columnaris* leaves in rodent.

## Material and Methods

*Collection and extraction of plant material*

The presence of flavonoids in *Araucaria columnaris* was reported in literature available for the pharmacological potential of the plant. This prompted us to select the plant for our study. The leaves of *Araucaria columnaris* were collected from the local places of Gwalior, Madhya Pradesh, washed with distilled water, shade dried and ground to coarse powder.

The leaf powder was defatted by shaking with petroleum ether. The defatted leaf powder was dried and extracted with 80% ethanol solution for 13 h using a soxhlet extractor. The solvent containing the soluble constituents was concentrated on thermostatic water bath to obtain the oleo-resinous extract. The extract was stored in desiccator to remove the excess moisture. The dried extract was weighed and used for further study.

*Preliminary phytochemical screening* (Shabbir et al., 2011; Tiwari et al., 2017)

The extract was screened for the presence of various secondary metabolites by qualitative tests. The presence of alkaloids was investigated using Mayer's, Wagner's, Hager's and Dragendorff's tests. Froth test, Borntragers test, Kedde's test and Keller-Kiliani test were employed for detection of saponins and other glycosides. Shinoda test, zinc hydrochloride reduction test and alkaline reagent test were used to confirm the presence of flavonoids in the extract. Gelatin, ferric chloride and Vanillin hydrochloride test were run for tannins and phenolic compounds in the extract. The extract was tested for sterols and terpenoids using Leiberman-Burchard and Salkowski test.

*Pharmacological Evaluation of extract*

The *in vivo* antidepressant action of the ethanolic extract of *Araucaria columnaris* (EEAC) was carried out in male albino mice weighing between 25–30 g by forced switn test (FST) and tail suspension test (TST) methods. The animal were grouped and housed in poly acrylic cages (38 x 23 x10 cm) in the animal house of the institute. Not more than four animals per cage were housed and maintained under standard laboratory conditions with natural dark and light cycle (14 h light/10 h dark) at 27±2°C and relative humidity (RH) 44-56% with free access to standard diet (Golden Feeds, India) and tap water *ad libitum* for one week for acclimatization before and during the experiments.

Animal were divided into 4 groups of 6 animals each for conducting the study. Group I was administered with normal saline and served as control, group II & III were administered 200 mg/kg (i.p) and 400 mg/kg of the EEAC respectively, whereas group V served as positive control and was administered with fluoxetine, 10 mg/kg (i.p).

*Forced Swim Test* (de Oliveira et al., 2011; Guan et al., 2013; Malviya et al., 2021)

The extract (EEAC) and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually placed in a glass cylinder (25 cm height, 10 cm diameter) filled with water (22-25°C) up to 10 cm height. Each mouse was allowed to swim for 6 minutes during the test, and the duration of immobility was observed and noted during the final 4 minutes of the test. The time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above water was regarded as the immobility period.

The animals were dried using tower and returned back to their housing conditions.

*Tail Suspension Test* (de Oliveira et al., 2011; Guan et al., 2013; Malviya et al., 2021)

The extract (EEAC) and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually suspended by tail using clamp (2 cm from the tip of the tail) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Minimal background noise was maintained and the testing was carried out in dark room. All animals were suspended for total 6 minutes, and the duration of immobility was observed and noted during the final 4 minutes of the test. Mice were considered immobile only when they hung passively and completely motionless.

The animals were used only once for this test.

*Statistical Analysis*

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad

Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The result were considered statistically significant when P- value less than 0.05 ($P<0.05$) vs control.

**Results and Discussion**

The branchlets of *Araucaria columnaris* grow in whorls and are having small green, spirally arranged leaves. The leaves appear as scales and are triangular in shape. The leaf bearing branchlets are narrow at the petiole, broaden towards the middle and narrow down towards the apex (Figure 1). Similar findings about the macroscopic characters of the plant are reported in literature (gardenia.net, 2021).

**Figure 1 Leaf of *Araucaria columnaris***

The extraction yield of the leaf using 80 % ethanol was found to be 36.9% and the extract was black in color.

The findings of the phytochemical analysis suggest the presence of cardiac glycosides, phenolics, terpenoids, sterols, and flavonoids in the leaves. The presence of tannins, abietanes (Aslam et al., 2013), and cardiac glycosides in *Araucaria columnaris* has been previously reported (Aslam et al., 2014).

*Antidepressant action*

The antidepressant action of the extract (EEAC) was tested using two animal models at two dose levels (200 and 400 mg/kg) and the results obtained are depicted in Figure 2 and 3. The immobility time and the swimming frequency was recorded and statistically analyzed using one way ANOVA followed by Dunnett's multiple comparison test.

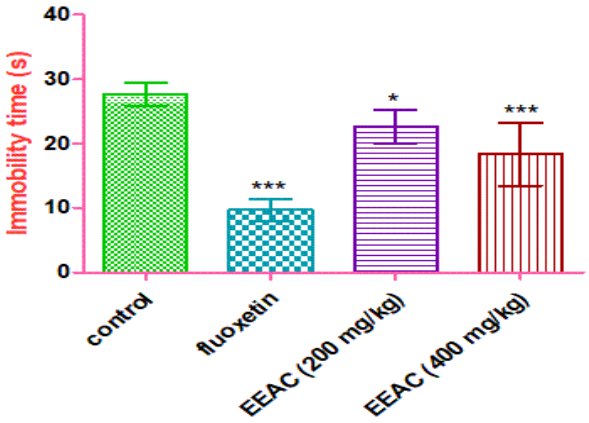


**Figure 2 Effect of EEAC (200 and 400 mg/kg) and fluoxetine (10mg/kg) on immobility time of mice in TST. *$p$<0.05, ***$p$<0.0001, Values are represented as mean ± SEM, ($n$ = 6)**

As it can be seen from Figure 2 that the immobility time for the compounds EEAC reduced significantly when compared to the control group. The immobility time for EEAC (400 mg/kg) was found to be 18.33 ± 1.994 sec and was significantly comparable ($p$<0.0001) to that of fluoxetine at a dose on 10mg/kg (9.67 ± 0.667 sec) (Table 2).

**Table 2 Immobility time of mice of various treatment groups in TST**

| Group | Treatment | Immobility Time (sec) |
|---|---|---|
| I | Saline | 27.67 ± 0.715 |
| II | Fluoxetine (10 mg/kg) | 9.67 ± 0.667*** |
| III | EEAC (200 mg/kg) | 22.67 ± 1.054* |
| IV | EEAC (400 mg/kg) | 18.33 ± 1.994*** |

*$p$<0.05, ***$p$<0.001 compared to control, Values are represented as mean ± SEM, ($n$ = 6)

**Table 3 Swimming frequency of mice of various treatment groups in FST**

| Group | Treatment | Swimming frequency |
|---|---|---|
| I | Saline | 17.33 ± 0.494 |
| II | Fluoxetine (10 mg/kg) | 43.00 ± 1.211*** |
| III | EEAC (200 mg/kg) | 25.17 ± 1.352*** |
| IV | EEAC (400 mg/kg) | 44.00 ± 1.317*** |

***$p$<0.001 compared to control, Values are represented as mean ± SEM, ($n$ = 6)

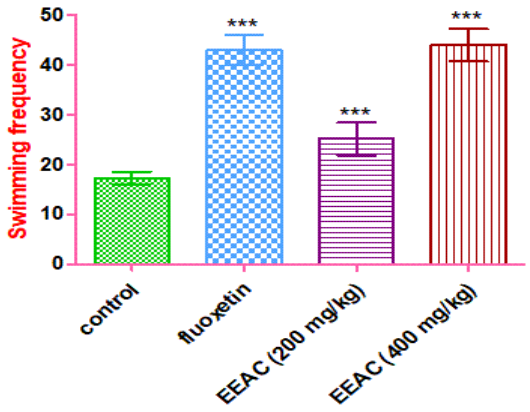


**Figure 3 Effect of EEAC (200 and 400 mg/kg) and fluoxetine (10mg/kg) on swimming frequency of mice in FST. ***$p$<0.0001, Values are represented as mean ± SD, ($n$ = 6)**

It was observed from the results that the swimming frequency increased significantly at both the dose levels of EEAC. The swimming frequency surpassed fluoxetine (43.00 ± 1.211) at 400 mg/kg dose of EEAC (44.00 ± 1.317) (Table 3)

As it can be seen from Figure 2 and 3 that the reference drug fluoxetine and the test extracts (EEAC) decreased the immobility of mice in FST whereas the swimming frequency was increased significantly.

## Conclusion

The present work was undertaken with an objective to establish the anti-depressant action of ethanolic leaf extract of *Araucaria columnaris*. The forced swim test and tail suspension test were used to establish the anti-depressant action. The presence of phenolic compounds could be responsible for the anti-depressant action of the plant. Fractionation and isolation of the components from extracts would be carried out in future to ascertain the responsible phytochemicals for the anti-depressant action.

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