# PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES ON THE LEAVES OF *COUROUPITA GUIANENSIS* AUBL.

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## Summary

Present work focused on carrying out the phytochemical and pharmacological studies on the leaves of *Couroupita guianensis* Aubl. Fractionation of petroleum ether extract of these leaves yielded three compounds. Compound 1, m.p. 79-81°C was identified as aliphatic hydrocarbon, while compound 2, m.p. 95-97°C was obtained in small yields and further work was not possible. Interestingly, compound 3, m.p. 273-75°C appeared to be a triterpene alcohol, according to its physicochemical and spectral data. Its lipid solubility and complex structure prompted us to undertake psychopharmacological studies in mice. Behavioral studies of compound 3 (1, 2.5, 5 mg/kg) using tail suspension test and despair swim test, suggested its potential antidepressant activity. The result of these tests revealed that efficacy of compound 3 at all doses was comparable to the standard antidepressant drug, imipramine (25 mg/kg). In conclusion, phytochemical and pharmacological studies on the leaves of *Couroupita guianensis* resulted in the isolation of compound 3, possessing antidepressant potential.

**Keywords:** *Couroupita guianensis*, phytochemical, psychopharmacological.

## Introduction

During the past decade, the indigenous or traditional system of medicine has gained importance in the field of medicine. In most of the developing countries, a large number of populations still depend on traditional practitioners, who in turn are dependent on medicinal plants, to meet their primary health care needs. The World Health Organization (WHO) has estimated that about three quarters of the world's population still relies on plant-derived medicines usually obtained from traditional healers, for their basic health-care needs (Kuruvilla, 2002; Farnsworth et al., 1985). Thus, it is clear that herbal medicine plays a pivotal role in therapeutic strategies in the modern world.

One such plant that has been used widely in traditional medicine is *Couroupita guianensis* Aubl. belonging to the family Lecythidaceae. It is widely cultivated for its large showy flowers and reddish - brown woody capsular fruits upto 20 cm in diameter. It is grown in Indian gardens as an ornamental tree. It is native to South India and Malaysia and is commonly known as Nagalinga pushpam in Tamil. Previous work on *C. guianensis* has shown that plant contains several chemical constituents with novel srtructures and possesses bio-active moieties. These include eugenol, linalool, fernesol, nerol, tryptanthrine, indigo, indirubin, isatin, linoleic acid, α, β- amirins, carotenoids, sterols (Wong and Tie, 1995; Rane et al., 2001; Bergman et al., 1985; Sen et al., 1974) and some acidic and phenolic compounds (Rajamanickam et al., 2009). Traditionally, the leaves of this plant have been used in the treatment of skin diseases (Satyavati et al., 1976), while the flowers are used to cure cold, intestinal gas formation and stomachache (Umachigi et al., 2007).

It has been reported that the petroleum-ether extractives of flower, leaves, bark and fruits of *C. guianensis* have promising antibacterial activity, particularly against gram negative *Salmonella typhi* NCTC 786 (Vahanwala et al., 2000). Methanolic extract (succ.) of *C. guianensis* leaves exhibited activity against both *Staphylococcus aureus* ACTC 3750 and *Salmonella typhi* NCTC 786. In all these extractives, MIC of 10 µg/ml was observed. In addition, studies have shown that petroleum-ether extract of *C. guianensis* flowers and methanol extract of fruits exhibited antimalarial and anthelmentic activities (Golatkar et al., 2001). Further, antimicrobial, wound healing and antioxidant potential of ethanolic extract of whole plant of *C. guianensis* has also been reported in literature (Umachigi et al., 2007).

Albeit, the known uses of the plant parts and their extracts in various disorders, especially those against microbial infections, none of the studies aimed at fractionating these extracts and studying the pharmacological profile of the isolated constituents. Hence, the present study focused on giving detailed description of phytochemistry of isolated components from the leaves of plant and studying the pharmacological profile of the isolated component using animal models.

## Materials and Methods

### Plant material

The leaves of *C. guianensis* (2 kg) were procured from the garden of L.T.M.G. College, Mumbai, India. The leaves were sun-dried and powdered (80-100 mesh) and stored in dry containers.

### Experimental animals

Swiss albino mice (20-22 gm) were housed under good hygienic conditions and standard conditions of temperature (25 ± 5ºC) with 12h/12h light and dark cycle in the departmental animal house. Animals were fed with standard pellet diet and had access to water, *ad libitum*. The experiments were performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of animal welfare division, Government of India, New Delhi.

### Extract preparation using different solvents

The dried milled leaf powder was extracted in a Soxhlet extractor with petroleum ether (60-80°C, 10 lit.), chloroform (8 lit.) and methanol (6 lit.) in succession. Pet-ether extract of *C. guianensis* yielded dark-brown oily residue (6.64 %); while successive chloroform and methanol extracts yielded (1.75 %) and (18-19 %) residues respectively.

### Fractionation using chromatographic separation

Petroleum-ether extract (10 g) was chromatographed over silica gel G (80-120 mesh, BDH 80) and the column was washed with pet-ether (60-80°C), benzene, chloroform and ethanol in succession. Eluted fractions were subjected to rechromatography. Pet-ether eluated fraction gave a white coloured compound (yield 4.36 gm). This on crystallization with methanol gave compound 1, m.p. 79-81°C. The chromatographic column on further elution with benzene gave two more compounds. Compound 2, m.p. 95-97°C was soluble in ethanol. Due to its low yield, further study was not possible. Another compound was obtained after exhaustive elution of the column with benzene. It was insoluble in ethanol. Repeated crystallizations from benzene (norit) furnished compound 3 in fine needles, m.p. 273-275°C (yield 177 mg). Attempts were made to characterize compound 3 using physicochemical tests and spectral studies.

**Assessment of antidepressant activity**

**Experimental groups**

Swiss Albino male mice were divided into six groups of six each. Group I animals served as untreated control. Group II animals were treated with reserpine (5 mg/kg, i.p.); served as negative control. Group III was treated with imipramine (25 mg/kg, p.o.); served as positive control. Group IV-VI received compound 3, intraperitoneally at a dose of 1 mg/kg, 2.5 mg/kg and 5.0 mg/kg respectively. The animals received test compound/std 30 min before the experiment.

**Tail suspension test**

The mouse was hanged for 6 min by clipping its tail (2 cm distant from the end) in a box (20×25×25 cm) with its head 5 cm above the bottom. The duration of immobility during the final 4-min interval of the test was recorded (Steru et al., 1985).

**Despair swim test**

The mouse was dropped into glass cylinder (20 cm in height) and 12 cm in diameter containing 8 cm deep water at 24–25°C and left there for 6 min. The duration of immobility during the final 4-min interval of the swimming test was measured (Porsolt et al., 1978). An animal was judged to be immobile whenever it remains floating passively in water in a slightly hunched but upright position, its nose just above the surface.

**Statistical analysis**

The results obtained in the present study were expressed as the mean ± SD. The numerical results were evaluated by application of one-way ANOVA with post Bonferroni test for multiple comparisons with the level of significance chosen at $p < 0.05$.

## Results

**Phytochemical and spectral studies**

Fractionation studies on the leaves of *C. guianensis* resulted in the isolation of three compounds. Compound 1 exhibited following spectral characteristics: m.p. 79-81°C, UV $\chi_{max}$ (MeOH) 209 nm (Є 1533), IR $\gamma_{max}$ (KBr): 2917 $cm^{-1}$ and 2849 $cm^{-1}$ (alkyl groups, C-H stretch), 1738 $cm^{-1}$, 1650 $cm^{-1}$ (aliphatic aldehyde/ketone), 1378 $cm^{-1}$, 1018 $cm^{-1}$, 887 $cm^{-1}$ (C-H bending). Mass spectrum of compound 1 showed peaks at 239, 218, 175, 135, 107 and 95 and molecular ion peak at 257. Compound 1, thus appeared to be an aliphatic hydrocarbon from spectral studies. Compound 2, m.p. 95-97°C was obtained in small quantities and further work on it was not possible. Compound 3 exhibited following spectral characteristics: m.p. 273-75°C, IR $\gamma_{max}$ (KBr): 3572, 2981, 2858, 1192, 1020 $cm^{-1}$, $^1$H-NMR (300 MHz, $CDCl_3$): δ 0.857 (3H, *s*), 0.989 (3H, *s*), 0.995 (3H, *s*), 1.005 (6H, *s*), 1.168 (3H, *s*), 0.936 (3H, d, J 6.9 Hz), 0.946 (3H, d, J 6.5 Hz) and 3.731 (1H, m). Mass spectrum of compound 3 showed retention time at 30.97 and 31.38 min and gave m/z 428 and 426 respectively.

**Tail suspension test**

The results of the tail suspension test showed that compared to reserpine-treated mice, compound 3-treated mice significantly ($p < 0.001$) shortened the duration of immobility in a dose-dependent manner. The efficacy of compound 3 was similar to that of standard drug, imipramine **(Table 1)**.

**Table 1: Effect of compound 3 treatment on mean immobility time in tail suspension test model in mice**

| Treatment | Dose (mg/kg) | Mean immobility time (sec) |
|---|---|---|
| Vehicle control | - | 69.5 ± 8.8 |
| Reserpine (negative control) | 5 mg/kg, i.p. | 170.3 ± 12.4 # |
| Imipramine (positive control) | 25 mg/kg, p.o. | 43.2 ± 5.2 *** |
| Compound 3 | 1.0 mg/kg i.p. | 44.0 ± 4.1 *** |
| Compound 3 | 2.5 mg/kg i.p. | 33.8 ± 4.3 *** |
| Compound 3 | 5.0 mg/kg i.p. | 26.3 ± 2.2 *** |

Values are mean ± S.D. (n = 6). #$p < 0.001$ significant as compared to vehicle control. Significantly different from negative control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), by one-way ANOVA followed by post Bonferroni test.

**Despair swim test**

Treatment with compound 3, also significantly ($p < 0.001$) reduced immobility time in mice, when compared to reserpine-treated mice **(Table 2)** in despair swim test model. The activity was again found to be dose-dependent and comparable to imipramine.

**Table 2: Effect of compound 3 treatment on mean immobility time in despair swim test model in mice**

| Treatment | Dose (mg/kg) | Mean immobility time (sec) |
|---|---|---|
| Vehicle control | - | 29.0 ± 2.5 |
| Reserpine (negative control) | 5 mg/kg, i.p. | 42.5 ± 5.0 # |
| Imipramine (positive control) | 25 mg/kg, p.o. | 19.7 ± 3.8 *** |
| Compound 3 | 1.0 mg/kg i.p. | 15.5 ± 2.9 *** |
| Compound 3 | 2.5 mg/kg i.p. | 11.8 ± 1.0 *** |
| Compound 3 | 5.0 mg/kg i.p. | 8.8 ± 1.6 *** |

Values are mean ± S.D. (n = 6). #$p < 0.001$ significant as compared to vehicle control. Significantly different from negative control (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$), by one-way ANOVA followed by post Bonferroni test.

## Discussion

Medicinal plants produce a diverse range of bio-active molecules, making them a rich source of different types of medicines. Herbal drugs in recent years have gained sufficient importance because of their efficacy and cost effectiveness and their ability to provide an effective alternative, especially for psychiatric patients with lingering conditions and intolerance to adverse effects of synthetic molecules (Zhang, 2004). Hence, medicinal plants with molecules that produce central nervous system modulating activities are increasingly attractive targets for the development of new drugs (Gomes et al., 2009).

A review of literature has shown that the biological studies on different parts of *C. guianensis* to be interesting. These studies gave promising leads for our present work. In the present study, three compounds were isolated from the leaves of *C. guianensis*. One of them, compound 3, m.p. 273-75°C was isolated in its pure crystalline form. Its solubility and crystallization from benzene (norit) in fine needles prompted us to study its characterization and activity. IR spectrum exhibited absorption bands at 3572 $cm^{-1}$, 1192 $cm^{-1}$ and 1020 $cm^{-1}$ which corresponds to OH group. The presence of CH stretch at 2981 $cm^{-1}$ and 2858 $cm^{-1}$ indicated it to be an aliphatic alcohol. NMR spectrum revealed six methyl signals at δ 0.857 (3H, *s*), 0.989 (3H, *s*), 0.995 (3H, *s*), 1.005 (6H, *s*), and 1.168 (3H, *s*) and two methyl doublets at δ 0.936 and δ 0.946. The presence of the quintet at δ 3.731 (1H, m) corresponds to 3 hydroxy secondary alcohol typical of either a sterol or triterpene alcohol. Since, compound 3, did not show positive test for sterol, it is suggested to be a triterpene alcohol. Mass spectrum of the sample showed presence of mixture of two compounds. The major compound (m.p. 273-75°C) showed molecular ion peak at 428 with retention time at 30.97 min and the minor compound showed molecular ion peak at 426 with retention time at 31.38 min.

The lipid solubility and complex structure of compound 3, prompted us to undertake its psychopharmacological studies in animal models. Depression is the most prevalent mental disorder and is recognized to be symptomatically, psychologically and biologically heterogeneous (Thase et al., 1995). In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem (Yu et al., 2002). Hence, the search for novel pharmacotherapy from medicinal plants that is safer and suitable for long-term treatment of psychiatric illnesses like depression.

Antidepressant activity potential of compound 3 was assessed using standard behavioral despair models. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the tail suspension test and despair swim tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants (Porsolt et al., 1977). Both these tests are the accepted stress models of depression. The immobility has been expected to reflect a state of 'behavioral despair and variants' or 'failure to adapt to stress' (Willner, 1991; Borsini et al., 1986). The behavioral despair exhibited by animals in turn can be correlated with depressive disorders in humans.

Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts in both these tests (Vogel, 1997). This decrease in duration of immobility is considered to have a good predictive value in the evaluation of potential antidepressant agents (Porsolt et al., 1977). Hence, in our present study, the significant ($p < 0.001$) reduction of immobility time shown by compound 3 treatment mice compared to reserpine control mice in both the tests, is suggestive of its antidepressant potential. The activity was found to be dose-dependent and comparable to standard drug, imipramine.

## Conclusion

In conclusion, phytochemical and pharmacological studies on the leaves of *Couroupita guianensis* resulted in the isolation of compound 3 (m.p. 273-75°C), a triterpene alcohol, possessing antidepressant potential. Further studies are warranted to elucidate the molecular mechanism of its antidepressant action and to explore other psychopharmacological activities of the compound.

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