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Original Research Article

Phytochemical analysis, Antioxidant and Analgesic Activity of Acetone Extract of *Curcuma Amada* Roxb Rhizome

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Abstract

Curcuma amada Roxb., often known as amba haldi, is a highly rhizomatic aromatic herb that contains a number of phytoconstituents. This study was done to investigate the antioxidant and analgesic properties of C. amada acetone extract. In-vitro antioxidant activity was assessed using the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay and the nitric oxide scavenging assay, while in-vivo analgesic activity was assessed using the tail flick method and the formalin induced pain test in male wistar rats weighing 150–200 gm at different doses (100 mg/kg, 200 mg/kg, and 400 mg/kg). According to the findings, the acetone extract of C. amada had antioxidant and analgesic efficacy at all three doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg as compared to the control group. C. amada acetone extract exhibited in-vitro antioxidant as well as in-vivo analgesic activity in experimental animals.

Keywords: Amba haldi; antioxidant activity; analgesic activity; acetone extract.

Introduction

Curcuma amada (mango ginger) is closely related to turmeric and it is a plant of the ginger family Zingiberaceae. The rhizomes *Curcuma amada* are morphologically similar to ginger but they have taste like to raw mango [1]. In traditional system of medicine like Ayurveda and Unani medicinal systems, mango ginger have much importance as an antipyretic, appetizer, alexiteric, diuretic, aphrodisiac, expectorant, laxative and emollient to cure biliousness, skin diseases, itching, bronchitis and inflammation due to injuries and asthma [2]. *Curcuma amada* have numerous biological activities include antibacterial activity, hypotriglyceridemic activity, antifungal activity, platelet aggregation inhibitory activity, anti-inflammatory activity, cytotoxicity, antiallergic activity, CNS depressant, brine-shrimp lethal activity, enterokinase inhibitory activity and analgesic activity [2-5].

The essential oil obtained from the rhizome has variety of therapeutic qualities and pharmacological activity [6]. As a result, the current investigation was carried out to assess the antioxidant and analgesic properties of *C. amada* rhizome extract.

Material and Methods

1 Collection of Plant Materials

In the month of March 2020, the rhizome of C. amada was purchased locally from the Karnal district of Haryana. Dr. S.S Yadav, Assistant Professor, Department of Botany, Maharshi Dayanand University, Rohtak, taxonomically identified and authenticated the plant specimen.

2 Processing of Plant Materials

The plant rhizomes were cleaned, rinsed with water, and then dried in the shade until all water particles were evaporated. The dried plant material (rhizome) was crushed into coarse powder after the rhizomes were chopped into little pieces.

3 Animals

Male Wistar rats were procured from Bilwal Medchem and Research Laboratory Pvt. Ltd, Jaipur's Animal House. Before starting the experiments, the animals were exposed to laboratory conditions for seven days. The animals were maintained at room temperature under 12 hour's light/dark cycle. The IAEC of the Bilwal Medchem and Research Laboratory Pvt. Ltd, Jaipur approved the techniques and experimental protocol used in this investigation bearing the reference number BMRL/IAEC/2020-64.

4 Preparation of rhizome Extract

300 g powder of C. *amada* was soaked in 900 mL acetone for 72 hours to obtain acetone extracts. Whatman No. 40 was used to filter the extract. The extracts were condensed in a rotary evaporator at 45°C at 200 rpm, and the residue being utilized for further analysis [7].

5 Phytochemical Analysis

Various standard methods [8-12] were used to analyze the presence of different bioactive components in *C. amada* acetone.

In-vitro study

1. Antioxidant activity by DPPH free radical scavenging assay

The DPPH free radical scavenging activity of *C. amada* rhizome acetone extract was determined by using the standard procedure described by Behera et al. [13] with slight modifications. The *C. amada* rhizome acetone extract was diluted with acetone to prepare different concentrations (10, 20, 30, 40, and 50 µg/ml). 1 ml of each of these different concentrations was mixed with 1 ml of DPPH (0.15 mM in methanol) and further, 1ml of solvent with 1 ml DPPH was taken as control and kept for 30 min. in dark at room temperature. Subsequently, the absorbance was recorded at 517 nm. The ascorbic acid and acetone were taken as standard and blank respectively in this study.

2. Nitric oxide scavenging assay

The nitric oxide scavenging activity of *C. amada* rhizome acetone extract was investigated spectrophotometrically [14]. The different concentration of acetone extracts (10-50 µg/ml) were taken in different test-tubes and the volumes was made up to 1.5 ml in each by adding sodium nitroprusside (5 mM) in phosphate buffer and was incubated at 25 °C for 30 min. After incubation, 1.5 ml of Griess reagent (1% Sulphanilamide, 3% Phosphoric acid and 0.1% Naphthyl ethylenediamine dichloride) was added to each test tube. The absorbance was recorded at 546 nm. Ascorbic acid was taken as reference standard for measuring the percentage of scavenging activity and it was measured as

$$\% \text{ Inhibition of Nitric oxide scavenging assay} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

Where, absorbance of control was the absorbance of the control (without extract) at 546 nm; Absorbance of sample was the absorbance in the presence of the extract at 546 nm. The experiment was done in triplicate.

***In-vivo* studies**

1. Tail flick test

The procedures described by D'Amour and Smith [15] with some modifications were used to analyze the tail flick test. Latency time was defined as the time taken by a rat to respond to focused heat stimulation (such as flicking or withdrawing their inflicted tail) (s). Rats were selected for the experiment using a sensitivity test, and those that did not attempt to remove their tail within 4 seconds were discarded. The rats were divided into two groups: control and test (n = 5). The acetone extract in dosages of 100, 200, and 400 mg/kg, pentazocine (30 mg/kg, orally), and vehicle (cosolvent) was administered orally.

The cut-off time was set to 10 seconds. After 30 minutes of administration of the test substances and 15 minutes of administration of pentazocine, the latency time was recorded every 15 minutes for 1 hour.

2. Formalin test

The procedures described by Dobuissonet al. [16] with some modifications were used to evaluate the analgesic activity of acetone extract of *C. amada*. For induction of pain, the rats were injected with 0.05 mL of formalin (2.5%) subcutaneously in the subplantar region of the right hind paw.

The rats (5 per group) were received *C. amada* acetone extract (100, 200 and 400 mg/kg, p.o), Aspirin (300 mg/kg, orally) and vehicle (cosolvent, p.o) respectively after 30 min of formalin injection. The time (s) spent for licking of the injected paw was considered as indicative of pain. The nociceptive responses were measured after the formalin injection for early phase (first 5 min) as well as for late phase (15–30 min).

Statistical analysis

In SigmaStat® version 3.5 Software, the statistical analysis was done using one-way ANOVA followed by Dunnett's multiple comparison tests. To demonstrate differences across groups, the results were reported as mean± S.E.M. The differences are considered significant when $p< 0.05$.

Results

1. Phytochemical Analysis

Results obtained for qualitative phytochemicals screening in rhizome of C. amada are presented in Table 1. Total thirteen phytoconstituents were screened out of which nine were found present in C. amada acetone extracts and rest other were absent. The phytoconstituents found present in the ethanolic extract are cardiac glycosides, carbohydrates, tannins, flavonoids, phenols, saponins, quinines, terpenoids and alkaloids which suggests that the rhizomes contain a wide range of phytochemical potential.

Table 1: Result of phytochemical evaluation of rhizome of *Curcuma amada.*

Sr. No	Chemical test	Results
1	**Cardiac glycosides:** Keller-Killiani test	+
2	**Tannins:** Foam test	+
3	**Phlobatannin:** Hydrochloric acid	-
4	**Saponins:** Frothing test	+
5	**Flavonoids:** Shinoda test	+
6	**Proteins:** Ninhydrin reagent	-
7	**Oxalates:** Ethanoic acid glacial reagent	-
8	**Alkaloids:** Dragendroff's reagent	+
9	**Sterols:** Liebermann-Burchard test	-
10	**Quinones:** Concentrated HCl Test	+

11	**Terpenoids:** Salkowki's test	+
12	**Carbohydrates:** Molisch's test	+
13	**Phenols:** Ferric chloride test	+

Key; + = present, - = absent.

Antioxidant activity of *C. amada*

1. DPPH free radical scavenging assay

The concentrations for both the standards and the sample ranged from 10 to 50 g/ml. According to the results of the study, the standard ascorbic acid has better antioxidant activity than *C. amada* acetone extract, (Figure 1). The antioxidant activity of *C. amada* extract may be related to the presence of higher phytoconstituents in the rhizome of *C. amada*. The standard sample was previously found to have stronger antioxidant activity than the methanolic extract of *C. amada* rhizome [17], which is similar to current result.

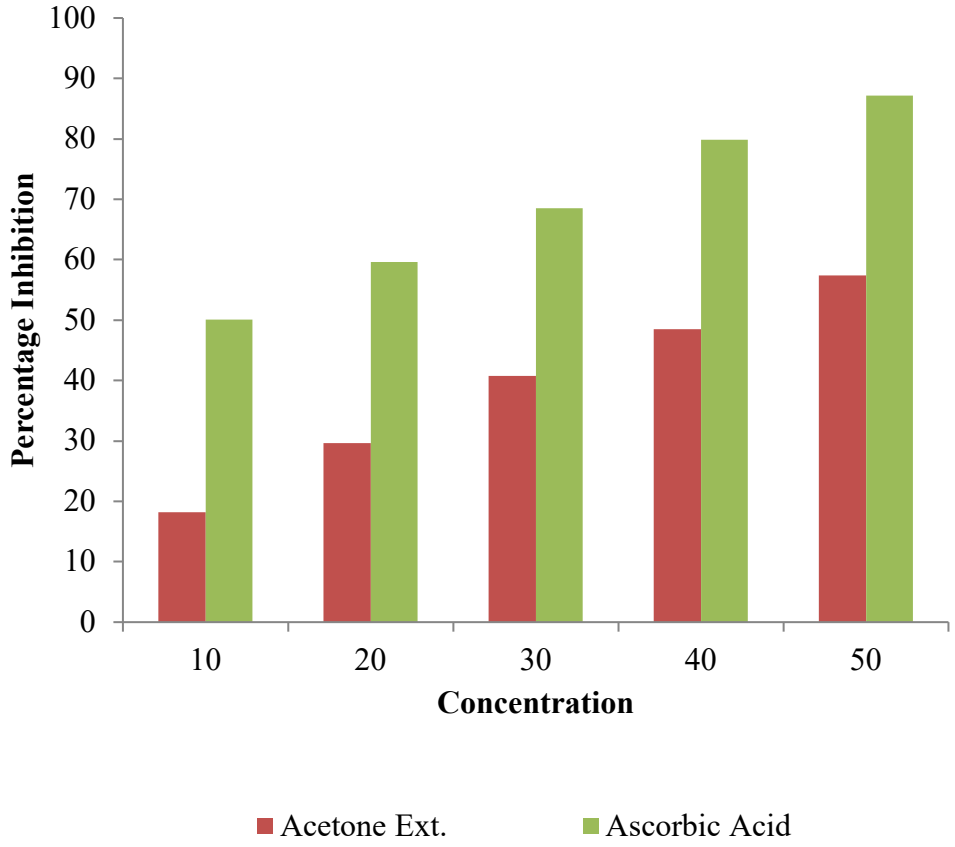

Figure1: DPPH Scavenging assay of acetone extracts of *C. amada* compared to ascorbic acid

2. Nitric oxide scavenging assay

Results indicated that the C. *amada* acetone extract increases the scavenging of nitric oxide in a dose-dependent manner as shown in Figure 2.

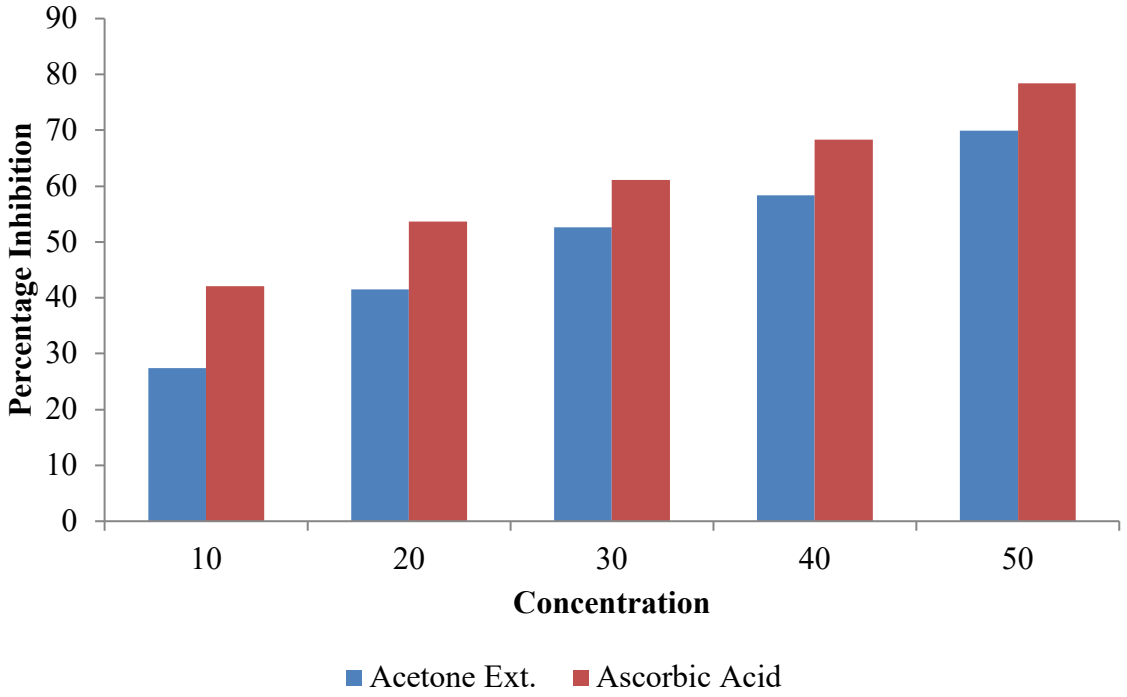

Figure 2: Nitric oxide scavenging assay of acetone extracts of *C. amada* compared to ascorbic acid.

***In- vivo* studies**

1. Tail flick test

The *C. amada* acetone extract had no significant antinociceptive effects on pain induced in tail flick in rats when compared to the standard drug pentazocine at any of the tested doses (100, 200, and 400 mg/kg) as shown in Table 2. As pentazocine is a centrally acting narcotic drug, resulted in significant increase ($p< 0.05$) in response time in tail flick test.[18].

Table 2. Effect of different doses of *C. amada* in tail flick test

Group	Reaction time in seconds			
	0 min	30 min	60 min	90 min
Control	4.01± 0.40	4.43±0.56	4.42±0.28	4.38±0.41
Standard	4.65± 0.42	6.13±0.48**	8.15±0.36*	7.50±0.37*
100 mg/kg	4.01±0.32	4.30±0.18	4.50±0.10	4.03±0.72
200 mg/kg	4.10±0.60	4.48±0.54	5.10±0.23	4.67±0.84
400 mg/kg	4.62±0.42	4.88±0.22	5.78±0.18	5.10±0.19

Value are expressed as mean±S. E. M **$p < 0.05$, *$p < 0.01$. Data were analyzed using ANOVA.

2. Formalin induced pain test

The formalin test is a pain model that predicts how an animal will react to constant pain caused by injured tissue [16]. The formalin test exhibits a biphasic peripheral nociceptive response that is divided into two phases: early and late.The neurogenic phase, also known as the early phase or tonic pain response, is directly triggered in the paw with the release of substance P. The production of histamine, prostaglandin, serotonin, and bradykinin during the late phase refers to the inflammatory pain response [19].

The *C. amada* acetone extract had analgesic effects in formalin-induced pain in both the early (0–5 min) and late (15–30 min) phases, as shown in table 3. In the early phase (neurogenic pain) and late phase (inflammatory pain), the treated groups showed substantial reductions in paw licking at dosages of 100, 200, and 400 mg/kg, respectively. When compared to the control, aspirin as an analgesic dramatically reduced paw licking in both the early and late phases.

In this experiment, the neurogenic pain (early phase) generated by formalin was minimized by *C. amada* acetone extract. Tail flick tests, on the other hand, are mediated by spinal nociceptive pathways, which differ from the neurogenic mechanism [15].

Table 2. Effect of different doses of *C. amada* in formalin induced pain test

Group	Paw licking Early phase (0-5 min)	Paw licking Late phase (15-30 min)
Control	63±0.36	79±0.79
Standard	47±0.50	33±0.86*
100 mg/kg	58±0.73	60±0.32
200 mg/kg	53±0.92	49±0.13
400 mg/kg	49±0.63	38±0.39**

Values are expressed as mean±S. E. M **p< 0.05, *p< 0.01. Data were analyzed using ANOVA.

Discussion

Synthetic medications are widely utilized for the treatment of a variety of ailments these days, but they come with many adverse effects and can cause plenty of health problems. Plants rich in bioactive compounds have been utilized extensively for the development of herbal medicine in traditional systems of medicine (Ayurveda and Unani) for a long time [20]. *C. amada* is a common spice in households and it possesses a number of biological and therapeutic characteristics [21].

The antioxidant activity of the *C. amada* acetone extract was studied by using two methods: the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging test and the nitric oxide scavenging test. Results indicated that the acetone extract exhibited antioxidant activity, which could be attributed due to the presence of various phytoconstituents as investigated by George and Britto[14].

Furthermore, the analgesic effect of acetone extract of *C. amada* was evaluated using analgesic animal models such as Tail flick and Formalin.

The highest reaction time in the tail flick method was 60 minutes for both the reference medication (pentazocine) and the *C. amada* acetone extract. The largest increase in reaction time for pentazocine was 8.15±0.36s at 60 minutes, whereas the maximum delay in reaction time for *C. amada* acetone extract was 5.78±0.18s at 60 minutes at a dose of 400 mg/kg. So on the basis of tail flick method, it is concluded that the acetone extract of *C. amada* has significant analgesic action as compared to control rats.

In Formalin induced pain test, at a dose of 400mg/kg, acetone extract of *C. amada* reduced paw licking in the early phase (neurogenic pain) and late phase (inflammatory pain) when compared to control animals, demonstrating its significant analgesic activity as well as its effectiveness in inhibiting pain-causing neurotransmitters.

The experimental evidence obtained in this work suggested that the acetone extract of *C. amada* dominates both central and peripheral antinociceptive activity, making it a dual pain

inhibitor. Carbohydrate, flavonoids, phenols, saponins, tannin, quinones, alkaloids, and terpenoid were identified in phytochemical investigations.

Many researchers have demonstrated the potential therapeutic efficacy and role of flavonoids in arthritis, cancer pain, colitis, osteoarthritis, and neuropathic pain [22]. Flavonoids are the predominant phytoconstituent contained in this extract. Mechanism of action of flavonoids is not fully understood, although research has revealed that flavonoids can be useful as analgesics, antioxidants, and anti-inflammatory drugs. Flavonoids, according to these researches, can inhibit the activation and production of a variety of cellular regulatory proteins such as cytokines and transcription factors, resulting in a reduction in cellular inflammatory responses and pain. In account of the above findings, the pharmacological effects of *C. amada* can be attributed due to the flavonoids contained in the plant [23, 24], which are responsible for the plant's analgesic and antioxidant activity.

Conclusion: Based on the data, it can be claimed that *C. amada* has high antioxidant and analgesic activity, which can be attributed to flavonoids present in plant.

Future Prospective: Mechanistic research is required to demonstrate medicinal potential of *C. amada* in humans.

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