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Study on phytochemical, antioxidant and analgesic activity of ethanolic extract of *Curcuma Amada* Roxb Rhizome

Vipin Saini
MM College of Pharmacy, Maharishi Markandshwar Deemed (To Be) University Mullana, Ambala-133207, Haryana
*Corresponding author email: anildaha@gmail.com
Orchid https: //orcid.org/0000-0002-1025-5109

Anil Kamboj
MM College of Pharmacy, Maharishi Markandshwar Deemed (To Be) University Mullana, Ambala-133207, Haryana

Abstract---Introduction: *Curcuma amada* Roxb. usually referred as ambahaldi is an exceptionally aromatic rhizomatic herb which contains number of phyto constituents responsible for several pharmacological activities. Present study is an attempt to explore antioxidant and analgesic activity of ethanolic extract of *C. amada*. Objectives: The goal of this work was to assess the antioxidant and analgesic activity of ethanolic extract of *C. amada*. Methods: For *in-vitro* antioxidant study, the DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging test and nitric oxide scavenging assay was used to assess the antioxidant activity while the *in-vivo* analgesic efficacy was evaluated by using tail flick method and formalin induced pain in experimental animals. Different doses of 100mg/kg, 200 mg/kg, and 400 mg/kg was used to evaluate analgesic activity of ethanolic extract of *C. amada*. Results: Ethanolic extract of *C. amada* exhibited significant antioxidant in DPPH and nitric oxide scavenging assay. 100 mg/kg, 200 mg/kg, and 400 mg/kg of ethanolic extract of *C. amada* produced significant analgesic activity in tail flick and formalin induced pain model in experimental animals. Conclusion: Ethanolic extract of *C. amada* exhibited significant antioxidant as well as i*n-vivo* analgesic activity in experimental animals.

Keywords---C. amada, ethanolic extract, antioxidant activity, analgesic activity.

Introduction

Curcuma amada Roxb. (Family: *Zingiberaceae*) is perpetual rhizomatic aromatic herb also known as Mango ginger and is available from November to April. Mango ginger (Curcuma amadaRoxb.) resembles ginger morphologically **[1].** *C. amada Roxb.* is a 60-90cm tall leafy tuft with petiolate, long, oblong-lanceolate, glabrous leaves that taper at both ends and are green on both sides.Flowers are clustered in spikes in the centre of the leaf tuft [2]. *C. amada* possess number of pharmacological activities like anti-inflammatory [3], antimicrobial activity [3], antifungal activity [3], hypotriglyceridemic activity [3], hypoglycemic[3] and anti-hyperglycemic[3] activity etc. The numerous pharmacologicalactivities of *C. amada*rhizome may be due to the presence of different bioactive compounds including curcumin, bis-demethoxy curcumin, demethoxy curcumin, phenol, and terpenoids etc. [4]. The essential oil extracted from the rhizome possesses different a number of pharmacological activities [5]. There are very few numbers of reports available on antioxidant and analgesic activity of *C. amada* till date. Therefore, the present study was carried out to evaluate the antioxidant and analgesic activity of *C. amada* rhizome extract.

Material and Methods

Collection of Plant Materials

The rhizome of *C. amada* was purchased locally from Karnal area, Haryana in March 2020. The plant specimen was taxonomically recognised and validated by Dr. S.S Yadav, Assistant Professor, Department of Botany, Maharshi Dayanand University, Rohtak.

Processing of Plant Materials

Fresh water was used to rinse and wash the plant rhizomes and then they were dried in the shade until all of the water content had evaporated. After drying, rhizomes were cut into small pieces and dried plant material was ground into coarse powder.

Animals

Male Wistar rats were procured from Bilwal Medchem and Research Laboratory Pvt. Ltd.'s animal house in Jaipur. Animals were exposed to laboratory conditions for seven days prior to the experiment. The animals were kept at room temperature in a light/dark cycle for 12 hours. The procedures and experimental protocol used in this work were approved by the IAEC of Bilwal Medchem and Research Laboratory Pvt. Ltd, Jaipur, under the reference number BMRL/IAEC/2020-64.

Preparation of rhizome Extracts by Solvent extraction

The extract of rhizome was made using the Soxhlet extraction technique. A thimble was filled with powdered plant material (50 gm in weight) and 250 ml of ethanol was used for extraction. The extraction process continues for 24 hours, or

until the solvent in the extractor's syphon tube becomes colorless. The extract was then placed in a container and heated at 30-40ºC on a water bath until all of the solvent had evaporated. The dried extract was stored in the refrigerator at 4ºC for future use in phytochemical analysis and other experiments.

Phytochemical Analysis

Different standard procedures were used to determine the presence of various bioactive components in the extracts [6-10].

In Vitro Study
DPPH free radical scavenging assay

The DPPH free radical scavenging activity was evaluated for the ethanolic extract of C. *amada* by standard procedure described by Behera *et al.* [11] with minor modification. The sample was made in different strengths (10, 20, 30, 40, and 50 µg/ml) by diluting the *C. amada* rhizome extracts in ethanol. Then, 1 ml of various concentrations of rhizome extract sample was blended with 1 ml of DPPH (0.15 mM in methanol) and 1 ml of solvent with 1 ml of DPPH was used as a control and maintained at room temperature for 30 minutes in a dark environment. After then, the absorbance was measured at 517 nm. In this study ascorbic acid was taken as standard and ethanol was employed as a blank.

Nitric oxide scavenging assay

The nitric oxide scavenging activity was investigated for the ethanolic extract of *C. amad* **[12].** The ethanol extract was placed in different test tubes at varied concentrations (10-50 g/ml), and the volumes in each test tube were increased to 1.5 ml by adding sodium nitroprusside (5 mM) in phosphate buffer. After 30 minutes of incubation at 25 °C, each test tube was filled with 1.5 ml of Griess reagent (1 percent Sulphanilamide, 3 percent Phosphoric acid, and 0.1 percent Naphthyl ethylenediamine dichloride). The absorbance at 546 nm was immediately recorded, and the percentage of scavenging activity was estimated using ascorbic acid as a reference.

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

Here Abs. of control and Abs. of sample are the absorbance of the control (without extract) and the absorbance of the sample (with extract) at 546 nm respectively. The experiment was carried out three times.

In Vivo Studies
Tail Flick Test

The tail flick test was evaluated using an analgesiometer and the procedures reported by D'Amour and Smith [13], with minor modifications. The latency time was described as the time taken for a rat to respond to intense heat stimulation (such as flicking or retracting its inflicted tail). For this experiment, the rats were

chosen by using a sensitivity test, and those who failed to retract their tail within 4 seconds were discarded. The rats were placed into two groups: control (n = 5) and test (n = 5). The standards drug pentazocine (30 mg/kg), C. *amada* ethanolic extract (100, 200, and 400 mg/kg) and vehicle (cosolvent) were administered orally to the rats. The cut-off time was set at 10 seconds. The latency time was recorded at every 15 minutes for 1 hour after 30 minutes of administration of the test drugs and 15 minutes of administration of pentazocine.

Formalin test

The method used to evaluate this study was slightly modified that was previously described by Dobuisson*et al.* [14]. For induction of pain the animals were administered 0.05 mL of formalin (2.5%) in the subplantar region subcutaneously of their right hind paws. Different doses of ethanolic extract of C. *amada* (100, 200, and 400 mg/kg), standard drug Aspirin (300 mg/kg,), and vehicle (cosolvent) were administered orally to the animals 30 minutes after formalin injection. For observation, rats were separated into different cages. It was considered that the amount of time spent for licking the injected paw was to be a sign of pain. The nociceptive responses were assessed in the early (first 5 minutes) and late (15–30 minutes) phases after the formalin injection.

Statistical Analysis

The statistical analysis was performed using one-way ANOVA and Dunnett's multiple comparison tests in SigmaStat® version 3.5 Software. The data were presented as mean± S.E.M. to show differences between groups, and when $p<0.05$, the differences were considered significant.

Results

Phytochemical Analysis

Table 1 shows the results of qualitative phytochemical screening in the rhizome of *C. amada*. The screening was done for thirteen phytoconstituents, ten of which were present in ethanol extracts while the rest were absent. Cardiac glycosides, phenols, carbohydrates, saponins, flavonoids, tannins, alkaloids, sterols, quinones, and terpenoids were found present in the ethanolic extract, suggesting that the rhizomes possess a wide range of phytochemical potential.

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

Sr. No	Chemical constituents	Tests/Reagents	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
DPPH free radical scavenging assay

The concentration ofstandard as well as sample were taken from 10 - 50µg/ml. After the experiment, it was found that standard ascorbic acid has higher antioxidant activityas compared to sample ethanol extractor rhizome of *C. Amada* (Figure 1). The presence of higher phytoconstituents in the rhizome of *C. amada* may be responsible for the antioxidant action of the extract. The results obtained are in accordance to result obtained by other researchers on *C. amada* [15] .

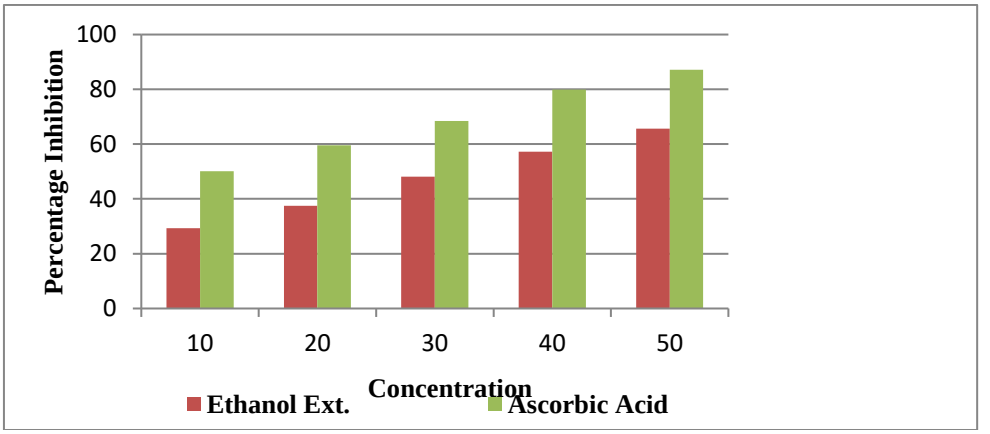

Figure 1. DPPH Scavenging assay of ethanol extracts of *C. amada* compared to ascorbic acid

Nitric oxide scavenging assay

As shown in Figure 2, the ethanolic extract of *C. amada* increases the nitric oxide scavenging in a dose-dependent manner.

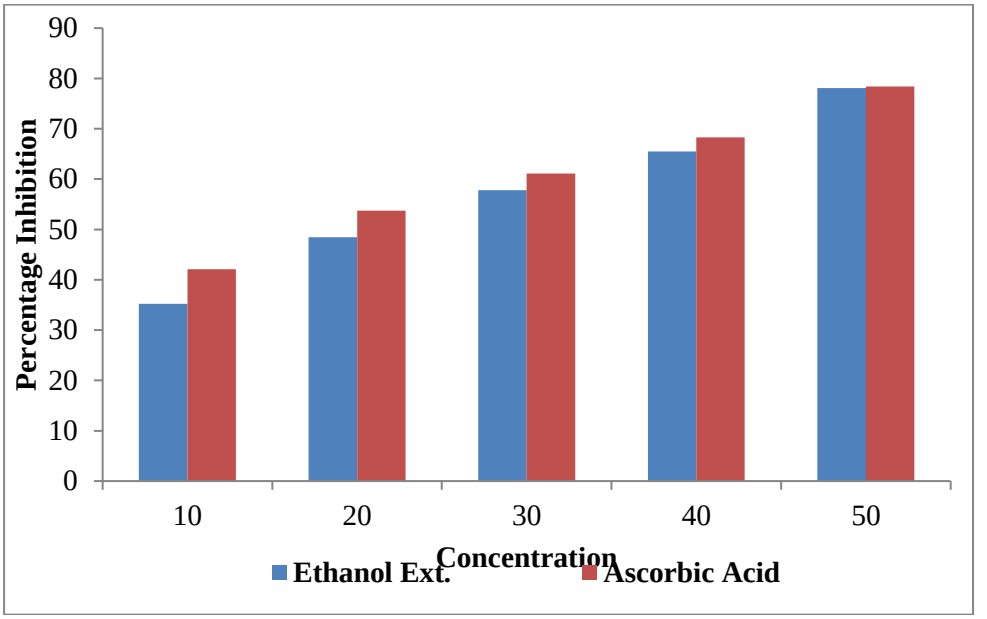

Figure 2. Nitric oxide scavenging assay of ethanol extracts of *C. amada* compared to ascorbic acid

In- Vivo Studies
Tail flick test

When compared to the standard drug pentazocine, the *C. amada* ethanolic extract showed significant antinociceptive effects on pain generated in tail flick test in rats, as indicated in table 2. The standard drug pentazocine is a narcotic drug which is centrally acting [16].

Table 2

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*
Test group 1	4.05±0.34	4.52±0.37	4.91±0.16	4.70±0.62
Test group 2	4.18±0.21	4.71±0.73	5.62±0.14	5.20±0.28
Test group 3	4.70±0.71	5.80±0.42**	6.75±0.40*	6.55±0.44*

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

Formalin induced pain test

The formalin experiment is a pain method for predicting how an animal will respond to a constant pain produced by tissue injury [14]. This method indicates the peripheral nociceptive responses in two phase ie. in early phase and in late phase. The early phase response is also known as neurogenic phase which is

directly triggered with the release of substance P in the paw while the late phase is also known as inflammatory pain phase which triggers with the release of prostaglandin, histamine, bradykinin and serotonin [17]. The ethanolic extract of *C. amada* demonstrated significant (p<0.05) analgesic effects on formalin-induced pain in both the early (0–5 min) and late (15–30 min) phases, as shown in Table 3. At doses of 100, 200, and 400 mg/kg, the treated groups demonstrated significant decreases in paw licking in the early phase (neurogenic pain) and late phase (inflammatory pain). In both the early and late phases, the standard drug aspirin significantly reduced paw licking when compared to the control.

Table 3 shows that the *C. amada* ethanolic extract had considerable ($p < 0.05$) analgesic effects on formalin-induced pain in both the early (0–5 min) and late (15–30 min) phases. In the early phase (neurogenic pain) and late phase (inflammatory pain), the treated groups showed significant reductions in paw licking at dosages of 100, 200, and 400 mg/kg, respectively. When compared to the control, aspirin as an analgesic significantly reduced paw licking in both the early and late phases. The ethanolic extract of *C. amada* was found to reduce the neurogenic pain (early phase) produced by formalin in this investigation. On the other hand the tail flick tests are mediated by spinal nociceptive pathways, which is different from the neurogenic pathway [13].

Table 3

Group	Paw licking Early phase (0-5 min)	Paw licking Late phase (15-30 min)
Control	63±0.36	79±0.79
Standard	45±0.50	23±0.86*
Test group 1	54±0.21	42±0.18
Test group 2	47±0.32	39±0.93**
Test group 3	43±0.18	28±0.72*

Each value is expressed as mean±S. E. M **p< 0.05, *p< 0.01. Data were analyzed using ANOVA.

Discussion

Nowadays, synthetic drugs are extensively used for treatment of different disorders but these chemically synthesized drugs have number of side effects and can arise many health-related complications. In traditional medicine system (Ayurveda and Unani) the plants which are rich in bioactive compound are extensively used for the preparation of herbal medicine from a long period[18]. In house hold *C. amada* is used as important spice and it also has several biological as well as medicinal properties [19]. The DPPH (2, 2-Diphenyl-1-picrylhydrazyl) free radical scavenging test and the nitric oxide scavenging assay were used to investigate the antioxidant properties of an ethanolic extract of *C. amada* rhizome. The ethanol extract exhibited the significant antioxidant properties, which might be attributed due to the presence of many phytoconstituents as investigated by George and Britto[12].

Due to the presence of antioxidant properties, *C. amada* can be used to cure major conditions like type 2 diabetes, infertility, and neurological disorders and can also be utilised in food preparation and preservation, as well as in the pharmaceutical industry [23]. Furthermore, the analgesic effect of Ethanolic extract of *C. amada* was evaluated using analgesic animal models such as the tail flick method and formalin induced pain method. In tail flick method both standard drug (pentazocine) as well as *C. amada* ethanolic extract showed maximum reaction time at 60 min. Pentazocine exhibited maximum increase in reaction time at 60 min which was recorded as 8.15±0.36s, similarly *C. amada* ethanolic extract showed maximum delay in reaction time of 6.75±0.40s at a dose of 400 mg/kg at 60 min. As compared to control rats, the ethanolic extract of *C. amada* exhibits significant analgesic action in the tail flick method.

In Formalin induced pain method an ethanolic extract of *C. amada* reduced paw licking in the early phase (neurogenic pain) as well as in the late phase (inflammatory pain) when compared to control animals at a dose of 400mg/kg, demonstrating its potent analgesic properties as well as its ability to block pain-inducing neurotransmitters. The results of this study revealed that the ethanolic extract of *C. amada* exhibits both central and peripheral antinociceptive activities which make it a dual pain inhibitor. Flavonoids' potential therapeutic effectiveness and role in management of colitis, cancer pain, osteoarthritis, arthritis, neuropathic pain, and cardiovascular illnesses have been already documented in several researches [20]. Falvonoids are the predominant phyto-constituent present in this extract. The exect mechanisms of action for flavonoids are unknown; however researches have shown that they can be used as analgesics, antioxidants, and anti-inflammatory agents. According to these studies, flavonoids can reduce cellular inflammatory responses and pain by inhibiting the activation and synthesis of a range of cellular regulatory proteins such as cytokines and transcription factors. In consideration of the above findings, it is concluded that the flavonoids found in *C. amada* are responsible for the plant's analgesic and antioxidant activity[21, 22] .

Conclusion

In view of above findings, it can be concluded *C. amada* possessed significant antioxidant and analgesic activity and these pharmacological activities are attributed to flavonoids present therein.

Future Prospective

Mechanistic exploration of *C. amada* is required to be carried out to prove therapeutic potential in human

Conflict of interest

Authors declare no conflict of interest

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