In vitro Antioxidant and In vivo Analgesic Activities of Citrullus lanatus Rind and Flesh Extract: A Comparison

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Abstract

The present study was carried out to assess and compare the analgesic and antioxidant potential of Citrullus lanatus rind and flesh extract. The methanolic extract of the rind and ethyl acetate extract of the flesh were evaluated for their in vivo analgesic activity through the acetic acid-induced writhing method on Swiss Albino mice. The free radical scavenging activity of the extracts was measured by total phenolic content measurement and the DPPH method. The flesh extract showed higher antioxidant activity than the rind extract in both methods. In case of the evaluation of analgesic activity, the results were obtained in a dose dependent manner. At 200 and 400 mg/kg doses, both extracts displayed moderate analgesic property in a statistically significant manner (p < 0.05) with respect to aceclofenac sodium. The flesh extract was found to provide a better effect than the rind extract in this case as well. It may be concluded that the ethyl acetate extract of C. lanatus flesh and the methanolic extract of the rind possessed potential antioxidant and analgesic activities and might be used as sources of nutraceuticals or functional foods.

Key words: Antioxidant, Analgesic, Citrullus lanatus, Comparison, Flesh Extract, Rind Extract.

Introduction

For thousands of years, products of natural origin have been considered as a great source of medicines and other medicinal agents and a huge number of modern drugs have been synthesized from natural sources mainly in traditional medicine (Alam et al., 2020; Harvey, 1999; Yuan et al., 2016). Based on the World Health Organization, over 80% of the population of the world relies on traditional medicine for their primary health care, with plant products having a significant impact on the remaining 20% (Pawar, 2014). Fruits and vegetable waste are also considered as a tremendous source of natural antioxidants and dietary fiber but there is a lack of information about their activity (Zeyada et al., 2008). It has been claimed that the functional properties of some peel components such as flavonoids, pectin, limonene, carotenoids and polymethoxyflavones should be considered (Al-Sayed and Ahmed, 2013). Flavonoids are a collection of polyphenolic compounds which can be found plentifully in the plant kingdom. In recent years, attention to the

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possible health advantages of flavonoids and other polyphenolic compounds has increased because of their potent antioxidant and free-radical scavenging activities (Rahman et al., 2013). Medicinal plants also have good potential as antimicrobial agents and it has become a crying need to discover new antimicrobial compounds with novel mechanisms of action to hinder the development of antibiotic resistance (Sekar et al., 2014).

Watermelon (Citrullus lanatus, Family: Cucurbitaceae) is a vine like flowering plant which is mainly endogenous in southern Africa and a significant crop in Africa and has the ability to cope in different environmental conditions (Adetutu et al., 2015; Mandel et al., 2005). It is a sprawling annual with coarse, hairy, pinnately lobed leaves and yellowish flowers. It is grown as edible fruit, which is commonly referred to as watermelon, and it is a special type of berry known as a pepo by botanists. It has a moderately hard rind which is generally green along with dark green stripes or yellow spots. Its interior flesh is succulent and sweet in taste which is normally deep red to pink in color, but can also be orange, white, or yellow, with numerous seeds. Watermelon can be divided into three main components as rind, flesh and seed. Flesh constitutes approximately 68% of the total weight, the rind approximately 30% and the seed 2%. Watermelon fruit consists of 91% water, 6% sugars and a low fat level (Rahman et al., 2013; Wehner, 2008; Zamuz et al., 2021). In a 100-gram (3.5 ounces) of mass, the fruit supplies 125 kilojoules of food energy and low amounts of other essential nutrients. Vitamin C is present in a considerable amount in 10% of the daily value. Carotenoids and lycopene are also present in an appreciable amount (Perkins-Veazie et al., 2006). Watermelon rind produces a good amount of amino acid citrulline (Rimando and Perkins-Veazie, 2005).

Watermelon seed has been evaluated as a potential source of anti-oxidant, anti-inflammatory and analgesic properties. It is also a great source of linoleic acid as a major fatty acid. Watermelon juice is used to keep liver, kidney and brain tissue safe from experimental tetrachloromethane toxicity (Messaoudi et al., 2019). There are also so many health benefits of watermelon. Studies showed that citrulline has the capability of expunging free radicals from the body by transforming itself into an amino acid which protects the body from the harmful effects of toxins. Researchers have claimed that citrulline helps to relax blood vessels and also can be used as a treatment for erectile dysfunction. In fact, it is capable of improving blood flow which has led to watermelon’s citrulline to be called as ‘Nature's Viagra’. Regular consumption of citrulline results in 30% less weight gain that helps to lessen muscle fatigue and therefore permits people to elongate their workout duration and manage their weight in a better way. Lycopene, an active ingredient of watermelon, can be a potential element to ward off prostate cancer which needs more studies to establish but diuretic attributes of watermelon help to keep the urinary tract in a healthy performing order (Aderiye et al., 2020; Erhirihe and Eken, 2013; Zamuz et al., 2021). Therapeutic effects of C. lanatus have been found which are related to its antioxidant property resulting from certain phytochemical compounds (Adetutu et al., 2015).

Materials and Methods
Collection of C. lanatus (watermelon): Watermelons are obtainable during the summer season throughout the whole country. The watermelon samples were collected from Shantinagar Bazar, Dhaka, Bangladesh.
Preparation of the extracts: To eliminate dust and other dirt from the sample, the fruits were washed with fresh water. Then it was sliced with a suitable clean knife. The seeds were collected manually and stored separately for further study. The flesh was cautiously scraped off and kept in a clean container. The rind portion was then chopped into small pieces and pureed and combined in a blender. The flesh portion of the sample was dried in an oven at 65°C for a week. Then the dried flesh (148 g) was macerated in ethyl acetate for a month. After that it was filtered and the filtrate was concentrated by evaporating the solvent by a rotary evaporator. The
concentrated ethyl acetate extract of *C. lanatus* flesh was kept in a beaker, labeled and stored for further use. The rind portion was also dried for a week using an oven at 65°C. Then the dried rind (241 g) was weighed and extracted with methanol. The methanolic extract was subjected to rotary evaporation and then kept in a container for further use.

*Experimental animal:* Swiss Albino mice (around 30 gm) were collected from Jahangirnagar University Animal House. The animals were 4-5 weeks old and of either sex. They were housed in standard polypropylene cages and kept at room temperature and relative humidity in a 12 hours light-dark cycle and were fed with standard diet and water *ad libitum.*

**Assay for antioxidant activity:**

(i) **Determination of total phenolic compounds:** Total phenolic content of the extracts was measured through an established method (Moniruzzaman *et al.*, 2019). Folin-Ciocalteu reagent was used as an oxidizing agent and ascorbic acid as the standard.

(ii) **Determination of free radical scavenging activity:** The antioxidant activity of the extract was checked using the established method involving 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Moniruzzaman *et al.*, 2019).

**Determination of analgesic activity:** The analgesic activity of the methanolic extract of *C. lanatus* rind and the ethyl acetate extract of the *C. lanatus* flesh was measured through the established acetic acid-induced writhing method (Razan *et al.*, 2016). This experiment was conducted *in-vivo* using Swiss Albino mice. Here, the negative control group was administered Tween-80 solution and the positive control group received Tween-80 solution with aceclofenac sodium orally. The experimental groups received the extracts at 200- and 400-mg/kg b.w. orally. Thirty minutes later acetic acid solution was administered intraperitoneally to each experimental animal. The percent (%) inhibition of writhing in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

\[
\% \text{ Inhibition} = \frac{(W_C - W_T) \times 100}{W_C}
\]

Where, \(W_C\) is the average number of writhing in control group and \(W_T\) is the average number of writhing in the test animal.

**Results and Discussion**

**Antioxidant activity:**

*Determining total phenolic compounds:* The antioxidant activity of plants is often observed as a result of various phenolic compounds, namely flavonoids, phenolic diterpenes and phenolic acids (Lopez-Martinez *et al.*, 2009). These compounds remove free radicals by the quenching mechanism and through the neutralization of peroxides and peroxy radicals (Michalak, 2006). It was found that both the flesh and the rind extracts of *C. lanatus* contained phenolic compounds and its level was higher in the flesh extract. The result can be seen clearly in figure 1.

![Figure 1. Total phenolic content of the rind and flesh extracts of *C. lanatus*.](image-url)

**DPPH free radical scavenging assay:** DPPH assay is another way to determine the antioxidant activity of a drug or an extract. It uses the compound named 2,2-diphenyl-1-picrylhydrazyl which stands for DPPH. The experiment revealed that the flesh extract showed an enhanced free radical inhibition effect than the rind extract. The IC\(_{50}\) values of the flesh and the rind extract were found to be 221.69 μg/ml and 277.30 μg/ml, respectively. Therefore, the flesh
extract of *C. lanatus* displayed better antioxidant properties than the rind extract, while both having moderate antioxidant properties. It can clearly be seen in figures 2 and 3. These findings matched well with the previous studies (Neglo *et al*., 2021; Zamuz *et al*., 2021).

![Figure 2. Plot of concentration vs % inhibition of standard (ascorbic acid), rind extract of *C. lanatus* and flesh extract *C. lanatus*.](image)

![Figure 3. IC\textsubscript{50} values of standard (ascorbic acid) rind extract of *C. lanatus* and flesh extract *C. lanatus*.](image)

**Analgesic activity:** Acetic acid-induced writhing in mice model was used to determine the analgesic activity of the extracts (Razan *et al*., 2016). Here, aceclofenac sodium in 50 mg/kg dose was used as the standard. Two doses (200 mg/kg and 400 mg/kg) were tested for each extract. The results are shown in tables 1-4.

### Table 1. Screening of the rind extract of *C. lanatus* for analgesic activity.

| Sample  | Dose (mg/kg) | Average writhing count | Writhing (%) | Inhibition (%) | p value  | Level of significance |
|---------|--------------|------------------------|--------------|---------------|----------|-----------------------|
| Control | 0.1 ml/10 g  | 38                     | 100          | -             | -        | -                     |
| Standard| 50           | 13.4                   | 35.26        | 64.74         | 0.0004   | Extremely significant |
| MG-1    | 200          | 26.8                   | 70.52        | 29.48         | 0.0104   | Significant            |
| MG-2    | 400          | 19.6                   | 51.57        | 48.43         | 0.0009   | Extremely significant |

MG stands for Mice Group

### Table 2. Screening of the flesh extract of *C. lanatus* for analgesic activity.

| Sample  | Dose (mg/kg) | Average writhing count | Writhing (%) | Inhibition (%) | p value  | Level of significance |
|---------|--------------|------------------------|--------------|---------------|----------|-----------------------|
| Control | 0.1 ml/10g   | 38                     | 100          | -             | -        | -                     |
| Standard| 50           | 13.4                   | 35.26        | 64.74         | 0.0004   | Extremely significant |
| MG-1    | 200          | 22                     | 42.10        | 57.90         | 0.0014   | Significant            |
| MG-2    | 400          | 19.6                   | 37.48        | 62.52         | 0.0012   | Very significant       |

MG stands for Mice Group.

In this experiment, the rind extract of *C. lanatus* at 200 and 400 mg/kg body weight showed 29.48% (p value 0.0104) and 48.23% (p value 0.0009) inhibition of writhing, respectively, compared to the standard aceclofenac sodium (64.74%) (p value 0.0004). The flesh extract of *C. lanatus* at 200 and 400 mg/kg body weight revealed 57.90% (p value 0.0014) and 62.52% (p value 0.0012) inhibition of
writhing, respectively compared to the standard drug. It can be concluded that the flesh extract possessed superior analgesic activity over the rind extract. Previous studies also support these findings (Erhirhie and Ekene, 2013; Neglo et al., 2021).

Conclusion

This study suggests that C. lanatus extract possesses potential free-radical scavenging- and analgesic activities. Since the crude extract of rind and flesh of C. lanatus demonstrated antioxidant and analgesic effects, it can be assumed that different active secondary metabolites are present in these extracts. This study can be a strong scientific evidence for this plant to be used as a valuable source of active constituents. However, further studies are necessary to elucidate the specific active compounds in the seed extracts of C. lanatus. It can also be said that, to understand the mechanism of action of antioxidant and analgesic activities, large scale experiments are needed.

Conflicts of interest

The authors declare no conflict of interest.

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