Research Article

Assessment of Chemical Composition, Analgesic and Anti-inflammatory Potential of the Methanolic Extract of *Citrullus colocynthis* (L.) Schrad

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ABSTRACT

Aim: The study was designed with an aim to determine the amount of total phenolics, flavonoids, condensed tannins, as well as to explore the analgesic and anti-inflammatory activity of the methanolic extract of the mature fruit of *Citrullus colocynthis* (L.) Schrad. **Materials and Methods:** The methanolic extract was tested for various bioactive phytoconstituents. The Total Phenolic Content (TPC), Condensed Tannins (CT) and Total Flavonoid Content (TFC) were quantified by Folin ciocalteu, vanillin assay and aluminum chloride methods respectively. Analgesic activity and anti-inflammatory activity were estimated by Eddy's hot plate and Carrageenan induced paw edema. **Results:** Bioactive compounds like alkaloids, flavonoids, phenolic acids, steroids, carbohydrates, proteins and reducing sugars were reported in extracts. The amount of condensed tannins, total flavonoid and total phenolic compound was found to be 168 µCT/g, 110.66 µg QE/g and 784 µg GAE/g, respectively. The analgesic activity of the higher dose of 500 mg/kg body weight and diclofenac sodium was maximum at 120 m after administration i.e. 8.56 sec and 8.92 sec respectively, while the lower dose exhibited a maximum effect of 7.54 sec at 60 m. The maximum percentage inhibition of extract (68.07%) at 500 mg/kg and ibuprofen (75.43%) was noted at 120 m of carrageenan injection. **Conclusion:** Results of our study affirmed that the methanolic extract produced analgesic and anti-inflammatory properties by virtue of the presence of bioactive components present in the extract. However, in order to establish its effectiveness, more research is suggested.

Keywords: Analgesic, Anti-inflammatory, Chemical composition, *Citrullus colocynthis*.

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INTRODUCTION

Herbs are sources of a wide gamut of biologically active components possessing an array of biological activities. The increased interest in the use of herbs as a source of medicine for treatment of diseases and disorders is evident with the research available in the literature. Plant metabolites which include phenolic acids, alkaloids, flavonoids, terpenes, steroids, glycosides, tannins etc. are of great scientific importance, having various beneficial properties. These act as antioxidants, anti-inflammatory agents, anthelmintic, analgesics, antivirals and antimicrobials.[1]

Injury, infection, cellular changes etc. may be factors causing inflammation which may be acute or chronic. Many of the non-steroidal anti-inflammatory compounds possess analgesic activity which facilitates recovery from injury, pain and disability. Analgesic and anti-inflammatory drug abuse is one of the major problems due to the over-the-counter availability of drugs. The development of synthetic drugs with more side effects has led to research on indigenous herbal plants with their negligible toxicity and low cost. Hence, exploring medicinal plants may provide a stepping stone for the discovery of safer and affordable agents.[2,3] The natural products serve as raw materials and therapeutic agents for traditional and modern medicine as they are

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rich sources of bioactive compounds. The secondary metabolites synthesized by plants may be used as a curative agent in therapeutics. Inflammation produces redness and soreness which may be due to infections, microorganisms, toxins or harmful chemicals. The inflammatory response is a protective process ,which removes damaged cells and helps in healing of the affected tissues.[4] Literature reports several herbs that exhibit anti-inflammatory or analgesic activity.[5,6]

Citrullus colocynthis, Arabic name 'handhal', belongs to the family *Cucurbitaceae*. It is also called the bitter apple. Several studies have been documented on the pharmacological effects like, hypolipidemia, anti-cancer, antioxidant and antidiabetic.[7,8] The various phytoconstituents reported from this plant include Catechin, quercetin, 5-@-stigmastin-3-beta-ol, p-cymene, cucurbitacin,, colocynthin, glucocucurbitacin I and -gluco-cucurbitacin, cucurbitacin L and elatericin B. Literature reports that these phytoconstituents possess medicinal properties such as anti-inflammatory, anti-ulcerogenic, anti-diabetic, anti-oxidant etc.[9] The current study was carried out to investigate the phytochemical composition and to assess the *in vivo* analgesic and anti-inflammatory of the mature fruits on experimental animals.

MATERIALS AND METHODS

Collection and preparation of plant

The plant was obtained from the Northern Border Province of Saudi Arabia. The plant was authenticated by Dr Samreen Somroo, Department of Basic Health Sciences and a voucher specimen PC-2023-06, has been placed in the College of Pharmacy, Northern Border University. The fruits were cleaned with water and dried in the lab, which were then pulverized to obtain course form of the powder and was used for research.

Extraction

The sample powder (100 g) was macerated in 200 mL of methanol for 24 hr in a closed container with occasional stirring. This was then filtered and concentrated using a rotary evaporator until a viscous consistency was obtained. The percentage yield was calculated using the following formula

$$\% \text{ yield} = \frac{\text{weight of the dry extract}}{\text{weight of the dry material}} \times 100$$

Qualitative analysis of the phytoconstituents

The preliminary qualitative study was performed to identify the phytoconstituents using standard methods.[10]

Quantitative Analysis for plant chemicals

Alkaloid, saponins, condensed tannin, total phenolic and total flavonoid content were estimated quantitatively by the methods available in the literature.[10-12]

Alkaloids Content

The sample was mixed with 10% acetic acid and placed at lab temperature for 4 hr. It was filtered and concentrated. A solution of ammonium hydroxide was added and was kept for 10 min for precipitation to take place. The residue thus obtained was then filtered, dried and weighed.

Test for Saponins Content

The powder was macerated using 10% aqueous ethanol in a water bath at 90º for approximately 2 hr. The mixture was added to diethyl ether with constant stirring. The diethyl layer was separated using a separating funnel and discarded. This was followed by the addition of n-butanol. The mixture was then washed with 5% sodium chloride and heated to dryness. The dried sample was weighted to get the saponins.

Total phenolic content

Folin ciocalteu method was utilized for total phenolic content estimation using APEL/PD-303UV spectrophotometer using gallic acid as the standard calibration curve for gallic acid was created using, aliquots of 100 µg/mL, 200 µg/mL, 300 µg/mL, 400 µg/mL, 500 µg/mL, 600 µg/mL and 700 µg/mL in methanol. Phenolic content was expressed as gallic acid equivalent. The Absorbance was recorded at 750 nm in triplicate.

Total flavonoid content

Aluminum chloride colorimetric method was used for this evaluation. Quercetin and the sample were prepared in methanol. A standard calibration curve was created using the required dilutions. TPC was expressed as Quercetin equivalent per gram. The measurement was repeated three times at 415 nm.

Condensed tannins

Vanillin assay was carried out using an APEL/PD-303UV spectrophotometer. A working reagent of 1% vanillin and 8% concentrated Hydrochloric acid was prepared and was used for the estimation. Absorbance was recorded (500 nm). A standard calibration curve was prepared using Catechin and the condensed tannin was calculated as the Catechin equivalent.

Analgesic activity

Analgesic activity was evaluated Eddy's hot plate.[12] Twenty-four rats were used in our experiment with an

average weight between 225-250. Standard conditions with free access to commercial pelleted rat food and water was maintained. Animal care, handling and usage were followed strictly as per the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals. A protocol was made to minimize the number of animals used. The experimental protocol was approved by the Institutional Animal Ethical Committee (SBRL/IAEC/17/22-23).

Experimental animals were separated into 4 different batches of six animals each. 1st group was control and was treated with 10 mL/kg of normal saline, 2nd group-standard diclofenac sodium (10 mg/kg body weight), 3rd and 4th groups used for the two doses of the extracts, i.e., 250 mg/kg (lower dose) and 500 mg/kg (higher dose) body weight. The lower dose and higher dose were selected based on a study reported in literature.[13] The extracts were administered orally using normal saline as the vehicle. After 1 hr of ingestion, rats were placed on Eddy's hot plate maintained at around 55-56ºC and the time duration taken for reaction (licking of the paws) to pain stimuli for each animal was recorded as the reaction time (sec) by using a stopwatch. The criterion for evaluation was withdrawal or licking of paws which was considered as the reaction time and this was recorded. The results obtained from the test sample were correlated with the standard drug.

Anti-inflammatory activity

Anti-inflammatory was determined by Carrageenan induced paw edema. Healthy animals of either sex were separated into groups. Group 1 was control and was treated with normal saline; group 2 was standard Ibuprofen, group 3 for low dose 250 mg/kg (extract) and group 4-high doses of 500 mg/kg (extract). Carrageenan (0.1 mL) was injected into the sub planter region of the right hind paw. Percentage inhibition was calculated using the formula;

$$\% \text{ of edema inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where, Vc-Volume of edema (control group) Vt-Volume of edema (treated group). Volume of the paw was measured at time intervals (0, 15, 30, 60 and 120 min) by Plethysmograph.

Statistical analysis

Data from the experiments was expressed as Mean±SEM. The results were evaluated by ANOVA followed Student's t-test *.p*-value less than 0.05 was used to express significance.

RESULTS

The extract obtained was pale green and had a yield of 20.9% w/w of dry sample. Preliminary analysis of the phytoconstituents revealed the presence of glycosides, alkaloids, flavonoids, phenolic compounds, steroids, proteins, carbohydrates and reducing sugars. The amount of alkaloids was found to be 3.5±0.48 in the methanolic extracts of the fruits, while the amount of saponins was found to be 1.66±0.29.

Folin ciocalteu method is a common method to estimate total phenolic content, hence it was used. Gallic acid was used as reference and a calibration curve was created using absorbance versus the various concentrations of gallic acid. The TPC in the fruit was found to be 784 µGAE/g, which was assessed from the linear regression equation (y=0.1608x+0.0548, R2=0.9979) as shown in Figure 1. Each point represents the mean of three experiments.

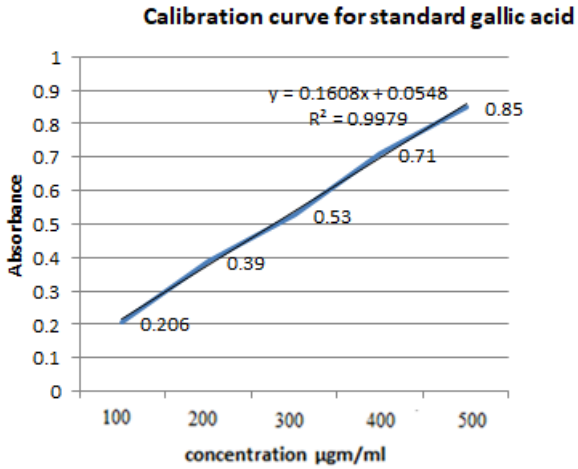

Figure 1: Calibration curve for standard gallic acid.

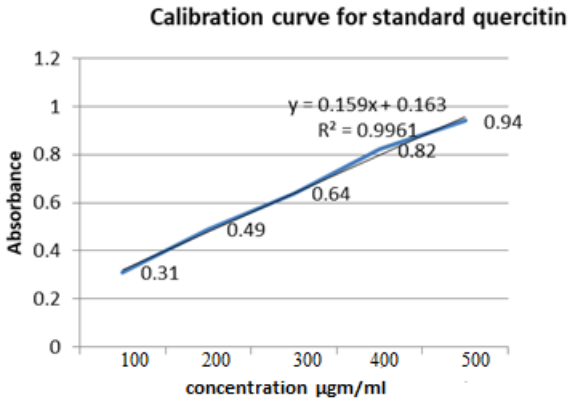

Figure 2: Calibration curve for standard quercitin.

Quercetin was used as the standard for determination of TFC. The standard solution was prepared by dissolving 25 mg of the quercetin in 25 mL of methanol as solvent

(1 mg/mL). The amount of TFC that was obtained from the methanolic extracts of the fruit was found to be 110.66 µgQE/g). This was assessed by the linear regression equation (y=0.159x + 0.163, R2=0.9961) as seen in Figure 2.

Results of the CT were evaluated from the linear regression equation obtained for standard Catechin (y=0.11x-0.014, R2=0.9974) as represented in Figure 3. The amount of CT was in the range of 168µCT/g.

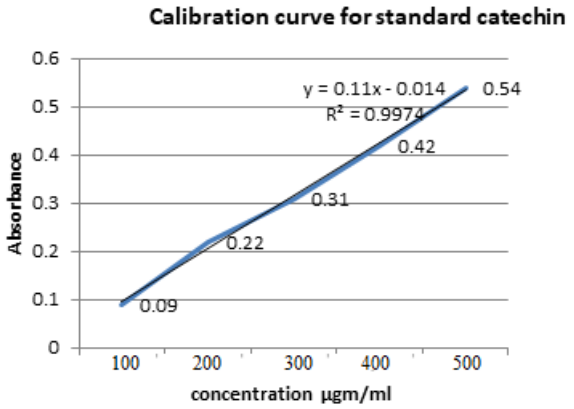

Figure 3: Calibration curve for standard Catechin.

The outcome of the analgesic activity of the extract is that; the extract showed significant analgesic activity by increasing the latency period to discomfort depending on the dose. At the higher dose, the activity was seen at 30, 60 and 120 min. The analgesic activity of the higher dose (500 mg/kg body weight) and standard drug was the maximum at 120 m i.e. 8.56 sec and 8.92 sec respectively, while the lower dose exhibited maximum effect of 7.54sec (p<0.05) at 60 m The standard and the higher dose increased the time significantly (p<0.001) when compared to the control. The central analgesic activity was evidenced by an increase in reaction time. The analgesic effect is as represented in Figure 4.

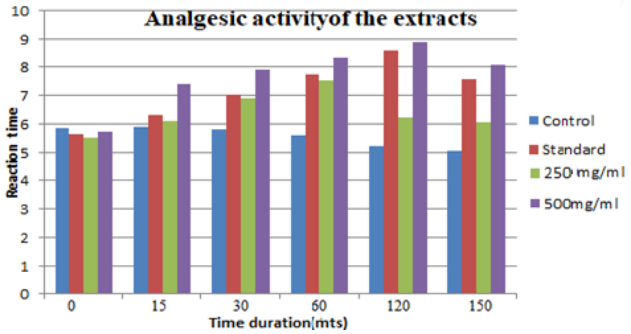

Figure 4: Analgesic activity by Eddy's hot plate method.

Anti-inflammatory properties of the extract were assessed by the carrageenan paw edema method and the results are as expressed in Figure 5. 500 mg/kg of the extract significantly (p<0.001) lowered the paw volume starting from 30 min after the administration of carrageenan with respect to control. The highest percentage inhibition of extract and standard was observed at 120 m i.e. 68.07% and 75.43% respectively.

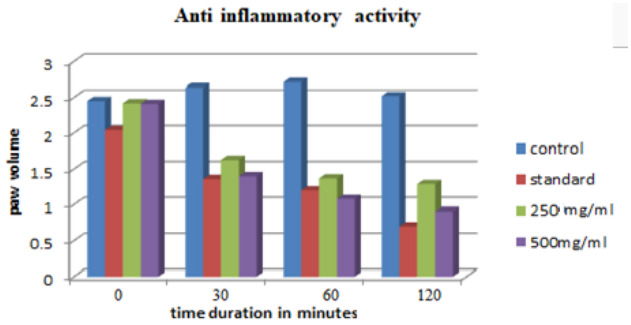

Figure 5: Anti-inflammatory activity of the methanolic extracts.

In both analgesic and anti-inflammatory activities, higher dose performed better when compared to the lower dose.

DISCUSSION

In our study, the mature fruit of *Citrullus colocynthis* was evaluated for qualitative and quantitive analysis of the phytoconstituents and also the analgesic and anti-inflammatory potential using standard methods. The ripe fruits were subjected to methanol extraction and the yield (20.9%w/w) was estimated. The extract was found to be rich in secondary plant metabolites such as alkaloids, glycosides, phenolics, flavonoid, terpenes, steroids, proteins and carbohydrates. Phenolic compounds and flavonoids possess numerous biological properties like antioxidant, anti-inflammatory, anti-allergic activity, antimicrobial, anticancer activity. They have been declared as important classes of phytoconstituents.[14,15] Hence, it was worthwhile to quantify the amount of TPC, TFC and CT in the plant extract.

Phenolic acids, hydroxy cinnamic acid derivatives and anthocyanins synthesized from shikmic acid and phenylalanine are important anti-oxidants and are powerful free radical scavengers and hence they exhibit anti-inflammatory effects.[16] Phenolic compounds exhibit anti-inflammatory activity by controlling different inflammatory markers like COX-2.[17] Therefore, the total phenolics with flavonoid content determined in this study were correlated with the anti-inflammatory property. Results of our study were in concurrence with reported literature.[17] Phenolic compounds are primary antioxidants or free radical scavengers present in plants. They are effective as oxygen scavengers,

reducing agents and hydrogen atom donators. Several methods are available for the evaluation of TPC but the Folin-ciocalteu method is one of the methods commonly used and is based on the reaction between phenolics and the Folin-Ciocalteu reagent which leads to the formation of blue complex of phosphotungstic-phosphomolybdenum.[18]

The TPC in the fruit was found to be 784 µgGAE/g. While the amount of condensed tannins and total flavonoids were found to be 168 µCT/g and 110.66 µgQE/g, respectively. Several studies have shown that flavonoids exhibit anti-inflammatory activity through various mechanisms, such as inhibiting prostaglandin synthesis, inhibiting phospholipase A2 enzyme, inhibiting COX-2 and 5-LOX, inhibiting histamine release from mast cells. Furthermore, it has been reported that tannins exhibit anti-inflammatory effects by virtue of their antioxidant properties which decreasing free radicals.[19]

Eddy hot plate method of evaluation was selected for evaluating central analgesic activity because it is the most common method due to its sensitivity and the tissue damage is negligible. Furthermore, the time required is less and the results are usually accurate. In this method, the threshold of pain of the experimental animal towards heat is measured.[20] Literature reports that the cyclooxygenase pathway advances the inflammatory pain via the conversion of arachidonic acid to prostaglandin E2.[21]

The analgesic effect exhibited was dependent on the dose. The extract at 500 mg/kg (higher dose) and the standard exhibited maximum activity at 120 m. The extract showed significant activity by raising the pain threshold beginning from 15 min until 120 m with respect to control. The highest analgesic activity was noticed for methanolic extract of higher concentration at all times during the experiment. The predicted mechanism of central analgesic activity could be due to the liberation of endogenous peptides. These endogenous peptides have been reported to hinder the pain impulses that are transmitted via peripheral mechanisms.[22,23] Carrageenan induced paw edema is the frequently used method for studying the anti-inflammatory activity of plant extracts.[24]

It is considered to be biphasic. Liberation of histamine as well as serotonin takes place during the initial phase, while the latter phase is due to prostaglandin and bradykinin release. It may be explained that the suppression of the first phase may be due to inhibition of histamine and serotonin, while the later phase may be due to inhibition of cyclo-oxygenase.[25] Edema is characterized by swelling and redness which are noticed instantly after administration of carrageenan.[26] The response to inflammation is determined by examining and analysing the difference in paw size on administration of the extracts. It can be suggested that the activity of this extract may be because of inhibition of prostaglandin and bradykinin.[27]

The methanolic extract at 250 and 500 mg/kg significantly reduced edema. The maximum inhibition by extract and diclofenac sodium was noticed at 120 m of carrageenan administration. The inhibition at 500 mg/kg of extract and standard ibuprofen was 68.07% and 75.43%, respectively. The various bioactive principles in the plant may be hindering the chemical mediators and bringing about suppression of the various steps involved in inflammation by inhibiting/impeding release of chemical mediators.[12] Methanolic extract of the plant exhibited significant analgesic as well as anti-inflammatory properties; this is attributed to the synergistic activity of the various phytoconstituents in the plant which has been proven by various researchers in the past. The phytochemical components such as flavonoids, alkaloids and phenolic compounds potently inhibit prostaglandins and other inflammatory mediators.[28]

A group of researchers has reported anti-inflammatory activity of various solvent extracts of immature seeds and fruits of *Citrullus colocynthis*.[29] Anti-inflammatory and analgesic potency seen in our research is an indication that inhibition of inflammatory mediator and release could be the mechanism of action and that the extract behaved like an NSAID due to the presence of both analgesic and anti-inflammatory effects. Furthermore, phytoconstituents such as flavonoids and phenolic compounds may play a major role in this activity.

CONCLUSION

Results of this investigation affirm that the mature fruit of *Citrullus colocynthis* demonstrated significant analgesic and anti-inflammatory activity and could be a potential anti-inflammatory and analgesic agent. It could be a suitable candidate for further investigation for use in rheumatoid arthritis. The results obtained give scientific proof and justify the traditional use of *Citrullus colocynthis* as an anti-inflammatory agent. Further research is required to identify the constituents that may actually be accountable for these properties.

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

SUMMARY

Herbs contain biologically active components possessing various biological activities and research on these herbs may provide a basis for the discovery of safe, affordable and effective agents. Results of our study affirmed that the methanolic extract of *Citrullus colocynthis* (L.) Schrad exhibited significant analgesic and anti-inflammatory activity in animal models and giving proof for its traditional use as an anti-inflammatory agent. This was attributed to the presence of the bioactive components such as phenolic acids and flavonoids present in the extract. Future research is required to explore the plant for identifying and isolating the bioactive components.

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