PHYTOCHEMICAL CONSTITUENTS AND INVITRO ANTI-INFLAMMATORY ACTIVITY OF AN IVY GOurd, COCCINIA GRANDIS

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ABSTRACT

Nature has provided lead molecules for the betterment of humans. In recent years pharmaceutical sector is focusing on development of new drugs and plant based drugs through investigation of leads from folkloric medicine which is being practiced for thousands of years. In India, Coccinia grandis (Cucurbitaceae) are used widely as vegetable. The objective of the present study is to investigate the phytochemical constituents and in vitro anti-inflammatory activity of the fruit extract to justify their use in folkloric medicine. Phytochemical screening of Coccinia grandis fruit powder in organic solvent (methanol) revealed the presence of alkaloids (0.22±0.07mg), flavonoids (1.09±0.00mg), saponin (0.05±0.01mg) and phenol (17.01±0.02mg). Anti-inflammatory activity was assessed by Human red blood cell membrane stabilization assay (HRBS) by varying concentration of the fruit extract ranging from 100mg/ml to 500mg/ml. The results obtained in the study showed that the fruit extract of Coccinia grandis showed 44.46±0.09% and 84.63±0.04% anti-inflammatory activity for 100mg/ml and 500mg/ml concentration respectively, and the activity was found to be concentration dependent and proved folkloric practice of the fruit extracts in curing various inflammatory responses.

Key words: phytochemical, pharmaceutical, folkloric medicine, anti-inflammatory.

INTRODUCTION

Natural products have long been a thriving source for the discovery of new drugs because of their chemical diversity. With increased use of herbal remedies, traditionally used medicinal
plants are receiving increased attention from scientific and pharmaceutical communities. The newer work on medicinal plants is mostly the rediscovery of traditional effects at cellular and molecular levels. *Coccinia*, a genus with about 30 species, *Coccinia grandis* (Cucurbitaceae) commonly known as Ivy gourd (Syn. *Coccinia indica*, *Cephalandra indica* Wight & Arnott.) is distributed in sub-Saharan Africa and South and Southeast Asia. *Coccinia grandis* is a climbing shrub with white flower. Every part of the plant exhibits pharmacological activity and finds application in treating various ailments in traditional Ayurvedic, Unani, Siddha practice. Leaves of the plant were reported to have anti-diabetic (Chopra et al., 1958), antibacterial activity (Girish and Satish, 2008), hepatoprotective activity (Rao et al., 2003). From fruit extract antioxidant activity has been reported (Venkatraman and Pari, 2003). Antispasmodic activity from the stem extract and anti-inflammatory activity from aqueous extract of fresh leaves have been reported (Junaid et al., 2009). It also found to be useful for treatment of  in bronchitis, and is effective for larvicidal action and antiulcer activity (Rahuman and Venkatesan, 2008). Major biochemical constituents such as alkaloids, carbohydrates, glycosides, flavonoids, tannins, saponins have been identified (Shaheen et al., 2009).

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants and commonly characterized by redness, swollen joint that is warm to touch, joint pain, its stiffness and loss of joint function. Inflammation is either acute or chronic. Inflammation is a self-defense reaction in its first phase, hence regarded as the main therapeutic target and often, the best choice to treat the disease and alleviate the symptoms. Thus, the present study is an attempt to analyze chemical compounds from fruit extract and to screen for their competence to modulate the expression of pro-inflammatory signals *in vitro* condition thereby assessing their capacity as anti-inflammatory agents.

**MATERIALS AND METHODS**

**Plant material and Sample Processing**

Fruits of *Coccinia grandis* were collected from local markets of Dindigul, Tamilnadu (India) during the month of December 2013. The sample was identified by Botanist, The Gandhigram Rural Institute – Deemed University, Dindigul, Tamilnadu (India). Samples were washed thoroughly in running water for removal of soil contaminants. After blotting the samples with tissue paper aseptically, samples were sliced into small pieces; dried in solar dryer (40°C) and
then ground to fine powder using mixer grinder. The powder samples were then stored in an air tight container in refrigerator for future use.

**Phytochemical Analysis**

**Solvent Extraction**

Sample powders stored in an air tight container in a refrigerator were taken and kept in hot air oven for 10mins to avoid any moisture if present. Twenty five grams of the dried powder were extracted successively with methanol over night using soxhlet apparatus. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator. The methanol extract was used for further phytochemical studies and *in vitro* studies.

**Estimation of Flavonoid**

Aluminium Chloride colorimetric method (Aiyegroro and Okoh, 2010) was used with some modifications to determine flavonoid content. 1ml of sample extract was mixed with 3ml of methanol, 0.2ml of 10% aluminium chloride, 0.2ml of 1M potassium acetate and 5.6ml of distilled water and remains at room temperature for 30mins. The absorbance was measured at 420nm. Quercetin was used as standard (1mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and expressed as quercetin equivalent (mg/g of extract).

**Estimation of Phenol**

Phenol was estimated by Singleton and Rossi (1965), method with slight modification. 1ml of sample was mixed with 1ml of Folin-Ciocalteau Phenol reagent. After 3min, 1ml of saturated sodium carbonate was added to the mixture and adjusted to 10ml with distilled water. The reaction was kept in the dark for 90min, after which the absorbance was read at 725nm. Gallic acid was used to calculate the standard curve (0.01-0.4 mM). Estimation of the phenolic compounds was carried out in triplicate. The results were mean values ± SD and expressed as milligrams of GAEs per gram of extract.

**Estimation of Saponin**

The method that was reported by Harborne (1973), was applied with some modifications. Sample of 20 g was weighed into a conical flask and added with 200 cm$^3$ of 20% aqueous ethanol. The sample was heated at 55°C for 4 hr in water bath with continuous shaking and then filtered off. The residue was rinsed with another 200 ml of 20% ethanol and filtered. The filtrate obtained was then concentrated to 40 ml using rotary evaporator. The concentrated
extract was transferred into a 250 ml separating funnel and added with 20 ml of diethyl ether before the mixture was vigorously shaken. The aqueous layer of solution was recovered while the ether layer was discarded. Afterward, the purification process was repeated twice using 60 ml of n-butanol. The purified sample was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated to dryness using rotary evaporator and dried in oven to a constant weight. The mass value was calculated as a percentage of saponin contain in sample.

**Determination of Alkaloids**

Alkaline precipitation gravimetric method described by Harborne (1973) for determination of alkaloid was followed. 10gm of the sample was dispersed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4h at 28°C. It was later filtered via Whatman No. 42 filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of Conc. aqueous NH₄OH until the alkaloid was precipitated. The precipitate was received in a weighed filter paper, washed with 1% ammonia solution dried in the oven at 80°C. The resulting weight difference gave the weight of alkaloid in the sample.

**Anti-inflammatory Assay**

Human red blood cell membrane stabilization method (Gandhidasan et al., 1991) was used for this study. The blood (10 ml) was collected from the healthy human volunteer who was not taken any NSAID’s for two weeks prior to the experiment. Blood was transferred aseptically to the heparinized centrifuged tube. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of isosaline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline. Various concentrations of extracts were prepared (100mg/ml – 500 mg/ml) using distilled water and to each concentration 1ml of phosphate buffer, 2ml isosaline and 0.5 ml of packed cell suspension were added and incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was read at 560nm. The percentage haemolysis produced in the presence of distilled water was taken as 100%. The percentage of HRBC membrane stabilization was calculated using the following formula.

\[
\% \text{ Membrane Stabilization} = \frac{100 - \text{OD of Extract treated sample}}{\text{OD of Control}} \times 100
\]
Statistical analysis
All experiments were conducted in triplicates and the parameters were given as means ± standard error. Both mean and standard deviation were performed where appropriate, using the statistical package within Microsoft® Excel Version 2007. Ink and the graphs were plotted using software Origin 6.0.

RESULT AND DISCUSSION
Traditional system of medicine consists of large number of plants with various medicinal and pharmacological importances and hence represents a priceless tank of new bioactive molecules (Clamp, 2007). Green vegetables occupy an important place among the food crops as they provide adequate amounts of many phytochemicals. The presence of secondary metabolites such as alkaloids, flavonoids, saponins, glycosides in the vegetables may contribute to their medicinal value (Schneider and Wolfing, 2004).

Thus in the present study the phytochemical constituents of the *Coccinia grandis* was analysed and the results was tabulated in Table:1. The phenol, flavanoid, alkaloid and saponin content of the *Coccinia* were found to be 17.01±0.02 mg/g, 0.22±0.07 mg/g, 1.09±0.00 mg/g and 0.05±0.01 mg/g respectively. The presence of phytoconstituents was in agreement with the earlier reports of Aritra Chatterjee and Sumana Chaterejee (2012). And also the quantitative analysis of the phyto constituents of the *Coccinia grandis* is well in concurrence with the earlier reports of Getachew et al. (2013).

*In vitro* anti-inflammatory activity of methanol extract of *Coccinia grandis* is given in Figure 1. The results revealed that all the concentrations of the extracts showed anti-inflammatory activity. However, the maximum activity was observed at highest concentration (500mg/ml) followed by lower concentrations such as 400 mg/ml, 300mg/ml, 200mg/ml and 100mg/ml respectively. The percentage anti-inflammatory activity of *Coccinia* ranged from 44.46±0.09% to 84.63±0.04% respectively. Ashwini et al. (2012), reported that *Coccinia grandis* exhibited 60-85% anti-inflammatory activity *in vitro*, the report was in agreement with the findings of this study. The results obtained were also similar to the reports of Majumder et al. (2008), who stated that the anti-inflammatory and antioxidant potency of the fruit extract highly reduces the risk of getting gastric ulcers.
Table:1 Phytochemical Constituents of *Coccinia grandis*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentration (mg/g)</th>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>0.22±0.07</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>1.09±0.00</td>
</tr>
<tr>
<td>Phenol</td>
<td>17.01±0.02</td>
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</tbody>
</table>

**CONCLUSION**

From the present study, it can be concluded that methanolic extract of *Coccinia grandis* fruit possesses more anti-inflammatory activity, which might be due to the presence of wide spectrum of phytochemicals. However the exact bioactive component and the mechanism involved in anti-inflammatory activity are yet to be elucidated. In depth scientific studies on *Coccinia grandis* fruits with *in vivo* and *in silico* models demonstrate the significance of the fruit as a potential anti-inflammatory agent.

**REFERENCES**


