

Research Article

ISSN 2320-4818
JSIR 2014; 3(1): 65-71
© 2014, All rights reserved
Received: 28-12-2013
Accepted: 26-01-2014

Sk. Amir Hossain

Biotechnology and Genetic
Engineering Discipline, Khulna
University, Bangladesh

Sr. N. Uddin

Montana State University, USA

Md. Abu Salim

Biotechnology and Genetic
Engineering Discipline, Khulna
University, Bangladesh

Razaul Haque

Biotechnology and Genetic
Engineering Discipline, Khulna
University, Bangladesh

Correspondence:

Sk. Amir Hossain

Biotechnology and Genetic
Engineering Discipline, Khulna
University, Bangladesh

Tel: +880-1720478775

E-mail: isti_99@yahoo.com

Phytochemical and Pharmacological screening of *Coccinia grandis* Linn

Sk. Amir Hossain*, Sr. N. Uddin, Md. Abu Salim, Razaul Haque

Abstract

Ethanol extract of *Coccinia grandis* Linn. (Family- Cucurbitaceae) were subjected to Phytochemical screening, which showed the presence of Alkaloids, Reducing sugar and Saponins. The pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine encouraged to check *Coccinea grandis* (Linn.) for possible antimicrobial, antidiarrhoeal and analgesic activities. The ethanolic extract at the dose of 500 mg/kg exhibited marked action against pain, ($p < 0.001$), percentage of protection was 60% while the standard drug diclofenac's percent of protection was found to be 76% at a dose of 50 mg/kg body weight. It showed no antidiarrhoeal activity at the dose of 500 mg/kg body weight. The obtained results provide a support for the use of this plant for traditional medicine and for its further investigation.

Keywords: Phytochemistry, Analgesic activity, Antidiarrhoeal activity, Pharmacology.

Introduction

Plants, the most wonderful gift from nature have been used as an origin of drugs. Various types of drugs are obtained from them. These types of plants are known as medicinal plants.¹ We use one or more of its organ for therapeutic purpose as a precursor of synthesizing of many useful drugs.² According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants.³

Coccinia grandis is a type plant belonging to the Cucurbitaceae (commonly known as gourd). It is commonly known as Telachucha, Tindora, Scarlet-fruited gourd and Ivy-gourd. It is natively found in India, Asia and Central Africa.¹ It is a climbing perennial herb which spread vegetatively or by seed. Seeds may be the valuable sources for oils and proteins which can cover both industrial and edible demand.⁵ The stem is an herbaceous climber or perennial slender climber with occasional adventitious roots forming where the stem runs along the ground. The tendrils are long, elastic with coil-like springy character that can wrap around the host to the entire length.⁶ The stem and root are the best used in skin disease, asthma, bronchitis, remove joint pains and many other. The most useful organ of these plants is leaves which are classified as palmately simple with five lobes while the shape varies from the heart to pentagon form.⁷ The leaves show anti-diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial⁸, and cathartic, expectorant activities⁴. The leaf constrain also found as hypoglycemic, hypolipidemic and antioxidant activity.⁹ The fruit of this plant is ovoid

in shape berry type which changes green to red color when become ripen.¹⁰ This part has also medicinal value in curing eczema, tongue sores and cerebral oxidative stress.¹¹

Coccinia grandis contain important raw material for drug production like bioactive compounds such as secondary metabolite like alkaloids, glycoside and saponin, b-amyryne, lupeol, cucubbitacin, cephalandrol, cephalandrine and flavonoids. By considering the significant of this plant, our work aimed at conducting phytochemical screening of this plant to identify the types of compounds present in the leaves and pharmacological studies for the screening of analgesic and anti-diarrheal activity in order to use this plant as a precursor of drug and others further investigated.

Materials and Method

To get the desired result, methods are needed for separation, purification and identification of many different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to the successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. As a result of modern extraction, and isolation techniques and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations.¹²

Collection of plant materials: The climber plant of *Coccinia grandis* were collected from the village Italy, Thana Shailkupa, and Jhenidah in March 2007.

Drying and Grinding

The collected plant parts (Aerial parts) were separated from undesirable materials or plants or plant parts. They were dried under sun for two weeks. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced. About 150 gm of powered material was taken in a clean, flat-bottomed glass container and soaked in 800 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. (Bibby RE200, Sterilin Ltd., UK).

Ethanol Extract

To get the solvent extract 100 gm dried sample (powdered form) was extracted in 500 mL of 95% ethanol for 7 days with periodic shaking and stirring. Then the entire mixing was accomplishing by filtrating via a funnel dipped in white cotton using Whatman filter paper number 1.

Evaporation of the Solvent

The filtrate (Ethanol extract) was obtained evaporated under ceiling fan until dried. It uses rotator evaporator to concentrate the resultant filtrate in the powdered form. It rendered a gummy concentrate of greenish color (Bibby RE200, Sterilin Ltd., UK). This gummy concentrate was designated as crude extract or ethanol extract and stored in refrigerator until further investigation.^{13, 14}

Chemical Group Tests

Testing of different chemical groups that present in the extract, represent the preliminary studies. The chemical group tests, which are performed as follows in each test 10% (w/v) solution of extract in methanol was taken unless otherwise mentioned in individual test.¹⁵

Test for Alkaloids

Mayer's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

Tests for Glycosides

A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water. Then, a few drops of aqueous sodium hydroxide were added. A yellow color is considered as an indication for the presence of glycosides.

Test for Steroids

Sulphuric acid test

1 ml solution of chloroform extract was taken and then added 1 ml sulphuric acid. Red color indicates the presence of steroid.

Test for gums

5 ml solution of the extract was taken and then Molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums and carbohydrate.

Tests for reducing sugar

Benedict's test

0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

Fehling's Test (Standard Test)

2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A and B & was boiled for few minutes.

Tests for tannins

Ferric Chloride Test

5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of Flavonoids.

Test for Saponins

1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

Screening for analgesic activity

Algesia (pain) is an ill-defined, unpleasant sensation, usually evoked by an external or internal noxious stimulus. Analgesics relieve pain as a symptom, without affecting its cause. They are used when the noxious stimulus (evoking the pain) cannot be removed or as adjuvant to more etiological approach to pain.¹⁰ Analgesic activity of drug or any test sample at different steps of pharmacological investigation can be assessed by acetic acid induced writhing method.

The acetic acid induced writhing method is an analgesic behavioral observation assessment method that

demonstrates a noxious stimulation in mice. The test consists of injecting the 0.7% acetic acid solution and then observing the animal for specific contraction of body referred as 'writhing'. A comparison of writhing is made between positive control (Diclofenac) and test sample given orally 30 minutes prior to acetic acid injection. The acetic acid is given intra peritoneally (IP). If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, i.e. the sample having analgesic activity will inhibit writhing. Diclofenac is used as reference standard drug. It has analgesic, antipyretic and anti inflammatory actions at different steps of pharmacological investigation with mild adverse effects. So the drug is used widely. Young Swiss-albino mice aged 4-5 weeks, average weight 20-28 gm were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR; B). They were kept standard environmental condition for one week for adaptation after their purchase and fed ICDDR; B formulated rodent food and water.

Antidiarrhoeal test

Diarrhea can be defined as frequent, often too precipitate passage of poorly formed stools. In, pathological term, it occur due to passage of excess water in faces.¹⁰ The method, described by Shoba and Thomas (2001), was followed for this study. The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. The positive control group received loperamide at the dose of 3 mg/kg orally; test groups received the EtOH extract at the doses of 500 mg/kg, body weight orally. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse, 30 minutes after the above treatments. During an observation period of 4 hr, the total number of faecal output and the number of diarrheic faeces excreted by the animals were recorded. A numerical score based on stool consistency was assigned as follows: normal stool=1 and watery stool=2.

Results and Discussion

Analgesic Test

The results of the test showed *Coccinea grandis* Linn ethanol extract of arial parts 500 mg/kg exhibit significant

($P < 0.001$), inhibition of writhing reflex by (60%), while the standard drug diclofenac inhibition was found to be 76 % at a dose of 25 mg/kg body weight (Table 1).

Table 1: Comparison of writhing of standard group, control group and test group

Animal group	Total Writhing	Mean Writhing	Standard deviation (SD)	Standard error(SE)	% Writhing	% Protection	T-test (value of p)
Control n=04	50	12.5	1.80	1.03	100		
Diclofenac sodium n=04	12	3	1.22	0.70	24	76	7.6 ($P < .001$)
Extract (500mg/kg) n=04	20	5	1.41	0.81	40	60	3.79 ($P < .001$)

Antidarrhoeal test

In the castor oil-induced diarrhea in mice, the ethanol extract of the leaf of *Coccinea grandis* Linn. at the doses of 500 mg/kg, reduced the total number of faeces as well

as of diarrhoeic faeces in a dose dependent manner, and the results were statistically insignificant (Table-2).

Table 2: Effect of *Coccinia grandis* Linn on the latent period of castor oil induced Diarrheal episode in mice

Group (Dose)	Number of Mice	Mean latent period (hr)	Standard Deviation (SD)	Standard Error (SE)	t-test (P-value)
I Control	1	0.76	0.069	0.034	
II Loperamide (50 mg/kg)	1	1.53	0.143	0.072	9.54 ($p < .001$)
Extract 250 mg/kg	1	0.81	0.08	0.04	0.925 ($p < .4$)

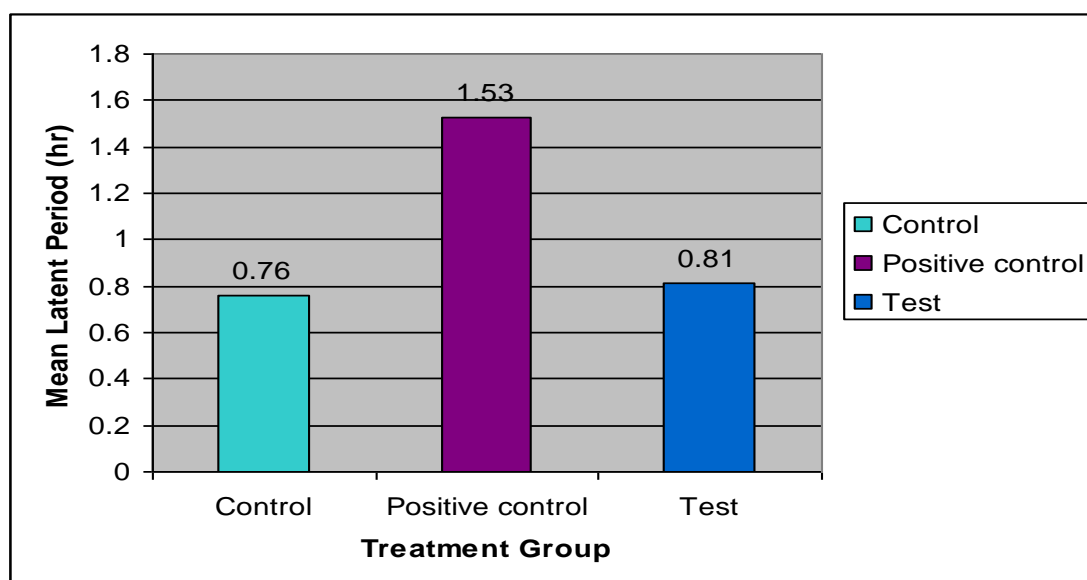


Figure 2: Effect of crude extract of leaves of *Coccinea grandis* Linn on castor oil induced diarrhoea of mice. Each bar represents mean latent period

Table 3: Effect of *Coccinia grandis* Linn on castor oil induced diarrhea in mice

Groups	Period of study (hr)	Mean latent Period \pm S.E. (t-test)	Mean no. of stools	S.E.	t-test (p value)
I Control	1	0.76 \pm 0.034	3.4	0.43	--
	2		6	.707	--
	3		7.6	0.43	--
	4		2	0.275	--
II Positive Control (Loperamide)	1	1.53 \pm 0.072	0	0	7.9
	2		1.2	0.255	6.39
	3		2.4	0.292	10
	4		1	0.447	1.90
III Ethanollic extract of <i>Coccinea grandis</i> 250 mg/kg	1	0.81 \pm 0.04	3.2	0.881	0.204
	2		4	0.894	1.75
	3		5.4	1.6	1.33
	4		1.3	0.538	1.16

Chemical Group Tests

The crude extracts *Coccinea grandis* Linn. Was subjected for chemical group tests and identified Alkaloids, Saponins and Carbohydrate Results of different group tests are given in table 4.

Table 4: Results of chemical group tests

Sample	Observation	Inference
Benedict's Test	Brick color precipitate was formed.	Presence of carbohydrate
Fehling's Test	Brick color precipitate was formed.	Absence of carbohydrate
Tests for Saponins	Foam was formed.	presence of Saponins
Mayer's Test	Yellow color precipitate formed.	Presence of alkaloid.
Dragendroff's Test	Orange brown precipitate formed.	Presence of alkaloid.
General Test	Immediate development of a red color is not occurred.	Absence of flavonoids.
Ferric chloride Test	Greenish black precipitate was not formed.	Absence of tannins.
Potassium dichromate test	A yellow precipitate was not formed.	Absence of tannins.
Test for Gums	Red violet color was not present.	Absence of Gums.

Conclusion

From the above data it has been seen that Ivy Gourd (*Coccinia grandis* Linn) has analgesic activity. It's controlling range 60% whereas standard sodium diclofenac control 76%. But it has been seen that Ivy Gourd (*Coccinia grandis*) has no antidiarrhoeal activity. In view of phytochemical screening some organic matters are present these are alkaloid, glycoside and saponin. So it can be said that ethanolic extract of Ivy Gourd (*Coccinia grandis*) has organic compounds which can show some pharmacological activity.

Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes Algesia by liberation of endogenous substances, which in turn excite the pain nerve endings.¹⁶ Increased levels of PGE2 and PGF2 α in the peritoneal fluid have been reported to be responsible for pain sensation caused by analgesic administration of acetic acid.^{17, 18} The ethanol extract of Ivy Gourd (*Coccinia grandis*) produced significant writhing inhibition comparable to the standard

drug diclofenac sodium. On the basis of this result it can be concluded that the ethanol extract of *Coccinia grandis* Linn. might possess analgesic activity.

In conclusion, it can be suggested that the ethanol extract of Ivy Gourd (*Coccinia grandis*) possess analgesic effects which support the traditional uses of this plant as antirheumatic. Therefore, further research is essential to find out the active principles responsible for these activities.

Acknowledgements

I thank Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh for supporting my research work in the field of pharmaceutical chemistry.

References

1. Yadav G., Mishra A., Tiwari A. Medical properties of Ivy Gourd (*Cephalandra indica*: a review. International journal of Pharma.Research & Development- online.2010; 2: 0974-9446.
2. Sofowara A. Medicinal Plants and Traditional Medicine in Africa, John Willey and Sons Limited, New York. 1982; 5: 55-56.

3. Myers. *Phytochemical methods (a guide to modern techniques to plant analysis)*. 3rd ed. Chapman and Hall. 1982; 335-337.
4. Chun M. E. Biology and host specificity of *Melittia oedipus* (Lepidoptera: Sessidae), a biological control agent of *Coccinia grandis* (Cucurbitaceae). *Proc.* 2001; 35: 41-45.
5. Jacks T. J., Hensarling T.P., Yatsu L. Y. Cucurbit seeds: I. Characterizations and uses of oils and proteins, a review. *Econ. Bot.* 1972; 26: 135-141.
6. Tindall H. D. *Vegetables in the tropics*. Westport, CT, AVI. 1983; 38.
7. Pekamwar S. S., Kalyankar T.M., Kokate S.S. Pharmacological Activities of *Coccinia grandis*: Review. *Journal of Applied Pharmaceutical Science*. 2013; 3: 114-119.
8. Dewanjee S., Kundu M., Maiti A., Majumdar R., Majumdar A., Mandal S. C.. In vitro evaluation of antimicrobial activity of crude extract from plants *Diospyros peregrina*, *Coccinia grandis* and *Swietenia macrophylla*. *Trop. Trop J Pharm Res* 2007; 6 (3): 773-778.
9. Tamilselvan N., Thirumalai T., Elumalai E. K., Balaji R., David E.. Pharmacognosy of *Coccinia grandis*: a review. *Asian Pacific Journal of Tropical Biomedicine*. 2011; 1(2): s299-s302.
10. Tripathi K. D., *Essentials of Medical Pharmacology*. 4th ed. New Delhi. Jaypee brother's Medical publisher. 2001; 17-26.
11. Umamaheswari M., Chatterjee T. K.. Effect of the fractions of *Coccinia grandis* on Ethanol Induced cerebral oxidative stress in rats. *Pcog Res* 2009; 1(1): 25-34.
12. Ghani, A. *Medicinal Plants of Bangladesh*. 3rd ed. The Asiatic Society of Bangladesh. 1998; 21:1-17.
13. Shaker M. Z. H., Hossain M. T., and Azam M. G.. Phytochemical Screening and Antidiarrhoeal Activity of *Hyptis suaveolens*. *International Journal of Applied Research in Natural Products*. 2012; 5: 1-4.
14. Pavia D. L., Lampman G. M., Kriz G. S.. *Introduction to organic laboratory techniques. A contemporary approach*, 2nd ed. Saunders Golden Sanburst Series, W.B Saunders Company, Philadelphia, London, Toronto. 1976; 599-614.
15. Evans, W. C. *Text book of Pharmacognosy*. 13th ed. University Press, Cambridge. Trease and Evan. 1989.
16. Taesotikul T., Panthong A., Kanjanapothi D., Verpoorte R., Scheffer J. J. C. Anti inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. *J. Ethnopharmacol.* 2003; 84: 31-33.
17. Derardt R., Jougney S., Devalce F., Falhout M.. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *Eur. J. Pharmacologic.* 1980; 51: 7-24.
18. Rang H.P., Dale M. M., *Pharmacology*, 2nd ed. Churchill Livingstone publisher, UK. 1993; 706-711.