Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2394-8234
JPP 2014; 3 (1): 222-225
Received: 29-03-2014
Accepted: 27-04-2014

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Phytochemical Characteristics, Antimitotic, Cytotoxic and Antiinflamatory Activities of *Coccinia grandis* (L.) J. Voigt

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ABSTRACT

Coccinia grandis (L.) J. Voigt, grows in the wild all over Bangladesh, is known to exhibit certain therapeutic properties. In the present work, leaf extract of *C. grandis* was considered for qualitative assessment of its secondary metabolite contents such as alkaloids, flavonoids, glycosides, saponins, sterol and tannins. Antimitotic, cytotoxic and antitumor activities of the ethanolic extract of leaf were also determined. Leaf extract showed strong inhibitory effect on seed germination, seedling's root growth of wheat and brine shrimp lethality with LC_{50} at 24.20 µg/ml. Antitumor assay showed inhibition of tumor induction on potato discs due to *Agrobacterium tumefaciens* by 66% at 1000ppm without any lethal activity against the bacterial agent. Results of anticarcenogenic activities of the leaf extract were discussed in relation to its secondary metabolites content.

Keywords: *Coccinia grandis*, Ethanolic Extract, Secondary Metabolites, Anticarcenogenic Activities.

1. Introduction

Traditional medicinal plants have been recognized for their therapeutic benefits for centuries. However, there is still lack of evidence for the clarification of their typical mechanisms of action. *Coccinia grandis* (L.) J. Voigt or Ivy gourd of Cucurbitaceae, an indigenous plant to Bangladesh and other South Asian countries, grows luxuriantly as tendril climber all over Bangladesh in the wild. *Coccinia grandis* leaf is traditionally used in anorexia, cough, diabetic, wounds and biliary-hepatic disorders. It has been reported to have antiinflammatory, antioxidant, antimutagenic, antimicrobial, antiulcer, hepatoprotective, expactorants and analgesic activities [1]. But the systemic effects and mechanisms of its anticancer properties like antimitotic, cytotoxic and antitumor activities have not been investigated in relation to its secondary metabolites content. Therefore, we aimed to investigate these properties in the present work along with the qualitative assessment of its secondary metabolites.

2. Materials and Method

Leaf extract of *Coccinia grandis* (L.) J. Voigt was used in the present work. For extraction, the collected tender leaf sample from a naturally grown plant was cleaned, chopped, air dried at room temperature and finally ground into coarse powder. About 100 g powder was macerated with ethanol (1:5) in a sealed container for 5 days at room temperature with occasional shaking. Extract was filtered through Whatman No.1 filter paper and evaporated to dryness under vacuum below 50 ºC to get about 3 g blackish extract. The extract thus prepared was kept at 4 ^{0}C for future use. Methods of Cromwell [2], Aplin and Cannon [3], Wall *et al.* [4], Farnsworth [5], Bhattacharjee and Das [6] were followed for qualitative assessment of different secondary metabolites. Alkaloid contents were assessed with the help of Dragendroff (D), Wagner (W), Mayer (M), Hager (H) and Tannic acid (T) reagents. The relative abundance of secondary metabolite contents in the extract was expressed by ─ or + signs, ranging up to 4+, signifying its abundance in degrees. Antimitotic activity of the leaf extract was determined according to Turker and Camper [7] using wheat seeds at germination and seedling growth stage. Cytotoxicity of the leaf extract was determined according to Meyer *et al.* [8] using brine shrimp nauplii and antiinflammatory activity on potato discs due to *Agrobacterium tumefaciens* was assessed according to Galsky *et al.* [9] using ethanol extract of leaf. Each set of experiment was replicated thrice.

3. Result and Discussion

The curative value of medicinal herbs largely depends on their secondary metabolites, especially alkaloids, terpenoids and phenolic compounds. Results given in Table 1 show that alkaloid test by all five reagents gave positive result in the order of 2+ to 3+ signifying its presence in the leaf in substantial quantity. Among the rests, all metabolites except resin showed positive result.

Table 1: Qualitative assessment of different secondary metabolites in *C. grandis* leaf.

Secondary metabolites										
Alkaloid Reagents used					Other secondary metabolites					
D	W	M	H	T	Flavonoid	Glycoside	Resin	Saponin	Sterol	Tanin
3+	3+	3+	2+	3+	+	+	-	+	+	+

The occurrence of different secondary metabolites suggests a wide range of biological application of the plant [10, 11, 12]. Several alkaloids like vinblastine, vincristine, camptothecin, taxol etc. are successfully employed in cancer treatment [13]. Kunle and Egharevba [14] suggested to consider the presence of flavonoids in a plant as indication of its antioxidant, antiallergic, antiinflammatory, antimicrobial and anticancer properties. However, glycoside derivatives showed very promising activity *in vitro* and *in vivo* [15] and two of them, ethylidene derivative etoposide [16] and theylidene derivative teniposide [17] were developed as anticancer drugs. Terpenoids and steroids are capable of preventing cancer [18], because of their anticarcinogenic effects [19]. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activities and can aid in wound healing and burns [20]. Recent reports show that tannins may have potential value as cytotoxic and/or antineoplastic agents [21]. Some researchers have also reported that some saponins have anticancer and immunemodulatory properties [14, 22]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions [23].

Growth inhibition test provides such way of detecting the antimitotic activity of chemical compounds under laboratory conditions. Antimitotic chemical compounds such as vinblastine and podophyllotoxin have been shown to inhibit cell division [24, 25]. Inhibition of cell division is a measure of the antimitotic activity of chemical compounds.

In the resent study, model of radish seed phytotoxicity assay described by Turker and Camper [7] was used to evaluate growth stimulation or inhibition properties of the ethanolic extracts of *C. grandis* leaf. The seed germination counts taken on the 5th day were 41.66±0.88 and 25.33±0.88 at 1000 ppm and 7500 ppm, respectively. (Figure 1). The root length measured on the 5th day of experiment was 13.56 mm±0.34 at 1000 ppm extract and 8.83 mm ±0.37 at 10,000 ppm extract (Figure 2). This indicates that higher doses were much more effective in inhibiting both seed germination and root growth in length. Similar result was observed by Inayatullah *et al.* [26] and Islam *et al.* [27].

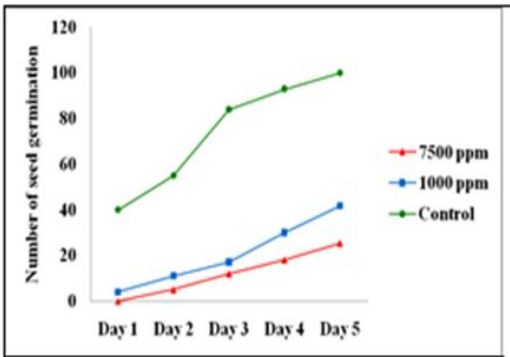

Figure 1 Antimitotic effects of ethanol extract of *Coccinia grandis* leaf as determined by assessing the rate of germination of wheat seeds

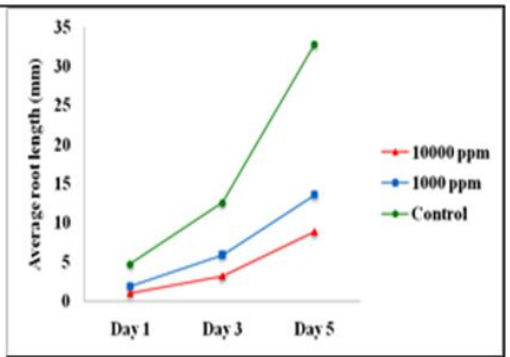

Figure 2 Effects of Ethanolic extract of *Coccinia grandis* leaf on the rate of root growth in length of wheat seedlings

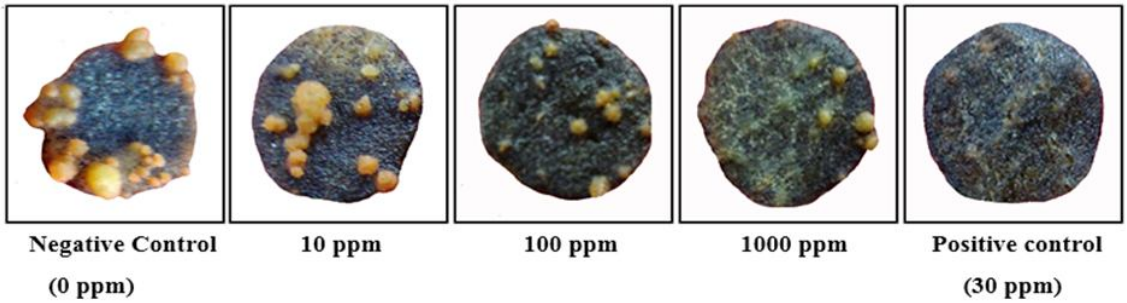

Fig 3: Effects of ethanolic extract of *C. grandis* leaf on crown gall tumor formation at different concentrations.

In the present work, *C. grandis* leaf extract showed LC_{50} value of 24.20 µg/ml with brine shrimp nauplii. The cytotoxicity of plant material would indicate the presence of antitumour compounds in plant extract [28]. Crude extracts resulting in LC_{50} values of less than 250 µg/ml could be considered significantly active and potential for further investigation [29]. The leaf extract in the present work, had shown LC_{50} value of less than 250 µg/ml and this might be the potential for inhibiting tumor or cancer cells. According to Coker *et al*. [30] antitumor agents might inhibit the initiation and growth of tumors in both plant and animal systems.

The crown gall tumor bioassay has helped in the short listing of plants with anticancer activity and in the discovery of novel compounds from plants [9, 31, 32, 33, 34]. It is a simple, inexpensive, rapid and sensitive assay for chemicals that disrupt the cell cycle (mitosis, S phase, etc.) regardless of their mode of action [30]. Inhibition of crown gall formation on potato discs by the leaf extract shows similar result against the P388 (3PS) leukemia in mice [32].

Table 2: Effect of *C. grandis* leaf extract on tumor formation activities on potato disces

Concentration	Mean number of Tumor ± SE	% of Inhibition
Negative control (Without extract or supressor)	25±0.7071	
10 ppm	19.6±1.0770	21.60
100 ppm	14±0. 9129	44.00
1000 ppm	8.5±0. 6455	66.00

The leaf extract of *C. grandis* of the present work exhibited a dose dependent manner of inhibitory action. The % inhibition of tumor (Photograph-1) was compared with the standard tumor suppressor drug, camptothecin (positive control), which completely inhibited the growth of gall tumor on potato discs. Highly significant tumor inhibition e.g. 44% and 66% was observed at 100 ppm and 1000 ppm concentration **(**Table 2**)** of the leaf extract respectively because plant extracts showing more than 20% tumor inhibition activity are considered significant [31]. Similar findings were also reported by others [28, 35, 36, 37] with other plants.

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