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Pharmacological activities of selected plant species and their phytochemical analysis

Maryam Jamil¹, Bushra Mirza^{1*}, Abida Yasmeen¹ and Mir Ajab Khan²

¹Department of Biochemistry, Quaid I Azam University, (45320) Islamabad, Pakistan.

²Department of Plant Sciences, Quaid I Azam University, (45320) Islamabad, Pakistan.

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In this study, four plants [*Chrozophora hierosolymitana* Spreng (Euphorbiaceae), *Ephedra gerardiana* Wall. ex Stapf (Ephedraceae), *Chrysanthemum leucanthemum* L. (Astraceae), and *Quercus dilatata* L. (Fagaceae)] collected from different regions of Pakistan were screened to identify any chemotherapeutic agents present in them. Seven methanol extracts of these plants (leaf, stem, and root extracts of *C. hierosolymitana*; stem and root extracts of *E. gerardiana*; aerial parts of *C. leucanthemum*, and aerial parts of *Q. dilatata*) were examined for cytotoxicity using brine shrimp assay, antitumor activity using potato disc assay, and phytotoxicity activity using radish seed bioassay. Two methanol plant extracts, that is, leaf extract of *C. hierosolymitana* and root extract of *E. gerardiana* showed significant brine shrimp cytotoxicity activity ranging from 171.55 to 523.8 ppm. Six of the seven extracts exhibited tumor inhibition at all the three concentrations tested, ranging from 10 to 80%. All extracts showed growth and seed germination inhibition at high concentration against radish seeds, while two extracts (root extract of *C. hierosolymitana* and aerial parts of *Q. dilatata*) showed growth stimulating effects at lower concentrations. Phytochemical tests showed the presence of alkaloids, saponins, anthraquinones, terpenoids, flavonoids, flavones, tannins, phlobatannins, and cardiac glycosides in different concentrations in these extracts.

Key words: Antitumor, cytotoxicity, plant extracts, plant growth regulatory factors, phytochemical constituents.

INTRODUCTION

The traditional medicinal methods, specially the use of medicinal plants, still play a vital role to cover the basic health needs in developing countries. Moreover, the use of herbal remedies has risen in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents having antitumor activity in animals as well as in plants (crown gall tumors). The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry has been possible, because of the large number of phytochemical and biological studies all over the world.

Four plants, *Chrozophora hierosolymitana*, *Ephedra gerardiana*, *Chrysanthemum leucanthemum*, and *Quercus dilatata*, were used in the present study. *C. hierosolymitana* belongs to family Euphorbiaceae. *Chrozophora* species are used in traditional medicine for the treatment of diverse ailments and also possess toxicity against rats (Adam et al., 1999). From the genus *Chrozophora*, the coumarin scopoletin, the alkaloid ricinine (Abdel-Sattar, 1985), flavonoids (Abdel-Sattar, 1985; Hashim et al., 1990), xanthenes, and chromones (Agrawal and Singh, 1988) have been reported. *E. gerardiana* belongs to family Ephedraceae. Members of this genus contain various medicinally active alkaloids (notably ephedrine) and they are widely used in preparations for the treatment of asthma and catarrh. The plant also has antiviral effects, particularly against influenza (Bown, 1995). *C. leucanthemum* belongs to

*Corresponding author. E-mail: drbushra@qau.edu.pk. Tel: +925190643007.

Table 1. List of plant species with respective plant extracts used for this study.

Plant species	Family name	Methanol extracts
<i>C. hierosolymitana</i>	Euphorbiaceae	<i>C. hierosolymitana</i> (leaf of <i>C. hierosolymitana</i>) (L) <i>C. hierosolymitana</i> (root of <i>C. hierosolymitana</i>) (R) <i>C. hierosolymitana</i> (stem of <i>C. hierosolymitana</i>) (S)
<i>E. gerardiana</i>	Ephedraceae	<i>E. gerardiana</i> (stem of <i>E. gerardiana</i>) (S) <i>E. gerardiana</i> (root of <i>E. gerardiana</i>) (R)
<i>C. leucanthemum</i>	Astraceae	<i>C. leucanthemum</i> (aerial part of <i>C. leucanthemum</i>) (A)
<i>Q. dilatata</i>	Fegaceae	<i>Q. dilatata</i> (aerial part of <i>Q. dilatata</i>) (A)

family Astraceae. Another member of the same family, *Chrysanthemum morifolium* is used frequently in traditional Chinese medicine. Historically applied to treat hypertension, angina, and fevers. Animal data suggest possible antiinflammatory and antipyretic activity (Huang, 1999). Some studies indicate that *Chrysanthemum* may have cytotoxic (Lee et al., 2001; Ukiya et al., 2002) and antibacterial activities (Urzua and Mendoza, 2003). Several pyrethroids, sesquiterpenoids, flavonoids, coumarins, triterpenoids, steroids, phenolics, purines, lipids, aliphatic compounds, and monoterpenoids have been isolated from different plant parts of *Chrysanthemum* (Kumar et al., 2005). *Q. dilatata* belonging to family Fegaceae is another plant species evaluated in the present study. The astringent effects of the *Quercus* were well known to the Ancients, by whom different parts of the tree were used, but it is the bark which is now employed in medicine. Its action is slightly tonic, strongly astringent, antiseptic, and can be used in the treatment of haemorrhages, chronic diarrhoea, dysentery, etc. It is a good substitute for quinine in intermittent fever, especially when given with chamomile flowers (Grieve, 1984). Different *Quercus* species contain shikimic acid (a cyclitol), methyl salicylate, and terpenoids (Evans, 2002).

As all the four plants selected for the present investigation belong to such genera whose several members are medicinal, therefore, these species were screened for pharmacological activities. In evaluating the pharmacological activities of a sample, to reach an appropriate conclusion, the use of a number of bioassays and careful comparison of all the data is required (Linton, 1983). In this study, the biological assays used to evaluate the biological activities of methanol extracts of the aforementioned plants were brine shrimp lethality test, crown gall antitumor assay, antibacterial assay, and reddish seeds phytotoxic assay.

MATERIALS AND METHODS

Plant and preparation of methanol extracts

For this study, four plant species of medicinal plants were collected

from different places of Pakistan (Table 1). These were identified following Flora of Pakistan and voucher specimens were deposited at the Herbarium of Department of Plant Sciences, Quaid-I-Azam University, Islamabad. The plant tissues were macerated at room temperature for two weeks with methanol and were filtered. The solvent was removed by rotary evaporation under reduced pressure and low temperature. Extraction of each plant part (leaf, stem, and root) was carried out separately and all necessary precautions were adopted to avoid cross contamination. All the extracts were stored at -20°C. The extract obtained from the leaves (200 g), stem (300 g), and roots (250 g) of *C. hierosolymitana* yielded 10, 2, and 8%, respectively. The extract obtained from the stem (166 g) and roots (56 g) of *E. gerardiana* yielded 18 and 10.5%, respectively. The extracts obtained from the aerial parts of *C. leucanthemum* (108 g) and aerial parts of *Q. dilatata* (741 g) yielded 6 and 13%, respectively.

Toxicity testing against the brine shrimp

The method used for brine shrimp lethality bioassay was as reported by Mclaughlin et al. (1991) and modified by Inayatullah et al. (2007). Brine shrimp (*Artemia salina*) eggs (Sera, Heidelberg, Germany) were sprinkled into a container containing 400 ml of artificial seawater, which was prepared with commercial salt mixture (HARVEST CO. H. K.) and distilled water. The container was left for two days under illuminated lamp. After this period, the eggs were hatched and second instar naupilii were observed swimming near the light source. Samples were prepared by dissolving 20 mg each of the extract in methanol (solvent) to make 2 ml (10,000 ppm) stock solution. From the stock solution, further concentrations (10,000, 1000, and 100 ppm) were made. A volume of 0.5 ml of the each concentration was taken in the vial. Solvent was allowed to evaporate overnight and seawater (5 ml) was added to each vial, resulting in final concentrations of 1000, 100, and 10 ppm, respectively. For negative control, 0.5 ml of methanol was used. As a positive control, MS-222 (tricaine methane sulfonate, Aquatic Eco-Systems, Inc.), a common fish anesthesizer, was used at concentrations of 1000, 100, and 10 ppm. Three replicates were prepared for each concentration. Ten shrimps were transferred to each vial. The vials were incubated for 24 h at room temperature under illumination. Survivors were counted with the aid of 3X magnifying glass after 24 h. The data was analyzed by probit analysis (Finney, 1971) for the determination of ED₅₀ (effective dose at which 50% shrimps killed) value of the extract.

Antitumor assay

The potato disc method was used for antitumor activity of plant

extract as reported by Ferrigni et al. (1982). *Agrobacterium tumefaciens* (At 10) was grown on Luria broth (Miller's LB broth) medium for 48 h at 28°C in shaking incubator. Sample was prepared by dissolving methanol plant extract (10, 1, and 0.1 mg) in 1 ml of dimethyl sulfoxide (DMSO) to make stock solution. From the stock solutions (10,000, 1000, and 100 ppm), further concentrations (1000, 100, and 10 ppm) were made by taking 0.5 ml of any of the stock solutions, 2.5 ml of autoclaved distilled water, and 2 ml of bacterial culture.

Negative control was prepared by taking 0.5 ml of DMSO instead of plant extract to prepare 5 ml of inoculum. Another control without *A. tumefaciens* culture was prepared by taking 0.5 ml of DMSO and 4.5 ml of autoclaved distilled water. Vincristine was used as positive control.

Red-skinned potatoes were purchased from a local market and surface sterilized by using 10% bleach solution. Cylinders of surface sterilized red skinned potato were made with the help of sterilized borer. The 5 mm thick discs of these potato cylinders were made and placed on solidified agar plates (10 discs per plate). Inoculum (50 µl) was poured on the surface of each disc of respective concentration as well as controls. The discs were examined after 21 days of incubation under dissecting microscope after staining with Lugol's solution (10% potassium iodide (KI) and 5% iodine in distilled water). The number of tumors per disc was counted. Percentage inhibition for each concentration was determined by using the following formula.

Percentage inhibition = $100 - \frac{\text{No of tumor with sample}}{\text{No of tumor with control}} \times 100$

Radish seed phytotoxicity assay

The method for phytotoxic assay was followed as reported by Turker and Camper (2002). For the phytotoxic assay, radish (*Raphanus sativus* L.) seeds were used and two different parameters were determined, namely, root length and percent of seed germination. Different concentrations were used for each parameter. Pure methanol was used as negative control. In the first experiment for the effect of test extract on radish seedling root length, four different concentrations (10,000, 1000, 100, and 10 ppm) were used. A volume of 5 ml of each concentration was added to sterilized 10 cm Petri plates containing a sterilized filter paper (Whatman No. 1). After vacuum evaporation of methanol, 5 ml autoclaved distilled water was added to each plate. Three replicates were prepared for each concentration. For control, 5 ml of methanol was used. Twenty sterilized radish seeds were placed in each plate. Petri plates were incubated in dark at 25°C. Root length was measured with the help of scale after five days.

In the second experiment for the effect of test extract on radish seedling germination, two different concentrations (7500 and 1000 ppm) were used. A volume of 5 ml of each concentration was added to sterilized 10 cm Petri plates containing a sterilized filter paper (Whatman No. 1). After vacuum evaporation of methanol, 5 ml autoclaved distilled water was added to each plate. Three replicates were prepared for each concentration. 100 sterilized radish seeds were placed in each plate. Petri plates were incubated in the dark at room temperature, $25 \pm 2^\circ\text{C}$. Germination was recorded every day from the 1st to the 5th day. Germinated seeds were removed from the plates and counted. The results were statistically analyzed using analysis of variance (ANOVA) and Duncan's multiple range test.

Antibacterial assay

Antibacterial assay was carried out as reported earlier (Ansari et al., 2005). Nutrient broth was inoculated with *A. tumefaciens* and was

incubated at 28°C for 24 h. One milliliter of this culture was added to 100 ml of molten nutrient agar cooled to 35°C, and was poured into Petri plates. Once the medium is solidified, wells were made with sterile cork borer (8 mm). A volume of 100 µl of the test sample (1000 ppm) and drugs, Roxithromycin and Cefixime-USP (1000 ppm) dissolved in DMSO was poured into appropriate wells. Pure DMSO was used as negative control. These plates were incubated at 28°C. After 24 and 48 h of incubation, the diameter of the clear zones showing no bacterial growth around each well was measured.

Phytochemical analysis

Qualitative phytochemical analysis of the seven crude extracts of the four plants was carried out by using standard procedures to identify the constituents as described by Edeoga et al. (2005) and Parekh and Chanda (2007).

Alkaloids

To identify the presence of alkaloids, 4 ml of 1% HCl was added to the 0.25 g of the plant extract, and then, it was warmed and filtered. To 1 ml filtrate, 6 drops of Mayor's reagents/Dragendorff reagent were added separately. Creamish precipitate/orange precipitate indicated the presence of respective alkaloids.

Saponins (Frothing test)

To detect saponins, 0.5 g of the plant extract was boiled in 5 ml of distilled water. After cooling it was shaken vigorously to produce stable persistent froth.

Anthraquinones

To check for the presence of anthraquinones, 0.5 g of the plant extract was boiled with 3 ml of 1% HCl and was filtered. To filtrate, 2 ml of benzene was added and was shaken well. The benzene layer was removed and few drops of 10% NH₄OH were added. Formation of pink, violet or red colour indicated the presence of anthraquinones.

Coumarins

For coumarins analysis, 0.5 g of moistened plant extract was taken in a test tube and covered with a filter paper moistened with 0.1 N NaOH. The test tube was placed, for few minutes, in boiling water. Then, the filter paper was removed and examined in ultraviolet (UV) light for yellow fluorescence to indicate the presence of coumarins.

Terpenoids (Liebermann-Burchard reaction)

To identify the presence of terpenoids, 0.5 g of the plant extract was dissolved in 2 ml of chloroform and was filtered. To filtrate, equal volume of acetic acid and a drop of concentrated H₂SO₄ were added. Blue-green ring indicated the presence of terpenoids.

Flavonoids and Flavones

To detect flavonoids and flavones, 0.5 g of the extract was washed with petroleum ether. The defatted residue was dissolved in 20 ml of 80% of ethanol and was filtered. The filtrate was used for the following test:

Table 2. Illustration of percentage mortality of brine shrimps at different concentrations of extracts and respective ED₅₀ value.

Methanol extract	Mortality of brine shrimps at concentrations (%)			ED ₅₀ (ppm)
	1000 ppm	100 ppm	10 ppm	
<i>C. hierosolymitana</i> (L)	80	30	20	171.55
<i>C. hierosolymitana</i> (R)	33.3	26.6.00	20	>1000
<i>C. hierosolymitana</i> (S)	36.6	20	20	>1000
<i>E. gerardiana</i> (S)	30	17	10	>1000
<i>E. gerardiana</i> (R)	53.3	40	13.3	523.8
<i>C. leucanthemum</i> (A)	50	-	0	1078
<i>Q. dilatata</i> (A)	40	20	10	>1000

1) About 3 ml of the filtrate was mixed with 4 ml of 1% AlCl₃ in MeOH in a test tube. Formation of yellow colour indicated the presence of flavanols and flavones.

2) About 3 ml of the filtrate was mixed with 4 ml of 1% KOH. A dark yellow colour indicated the presence of flavonoids.

Tannins

To test for tannins, 0.25 g of plant extract was boiled in 10 ml of distilled water and was filtered. Then, 1% FeCl₃ was added to the filtrate. Brownish green or a blue-black colouration indicated the presence of tannins.

Phlobatannins

Deposition of a red precipitate when 0.25 g of plant extract was boiled with 5 ml of 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Cardiac glycosides (Keller–Kiliani test)

To detect cardiac glycosides, 2 ml of glacial acetic acid and few drops of 1% FeCl₃ were added to 0.5 g of the plant extract. Then, it was underlaid with 1 ml of concentrated H₂SO₄. Green-blue color indicated the presence of cardiac glycosides.

RESULTS

Brine shrimp cytotoxicity assay

Three of the seven extracts *C. hierosolymitana* (L), *E. gerardiana* (R), and *C. leucanthemum* (A) exhibited potent cytotoxicity in brine shrimp cytotoxicity assay. ED₅₀ in these extracts was quite good ranging from 171.55 to 1078 ppm (Table 2). Results for percentage mortality of brine shrimp indicate that highest percentage mortality was observed at 1000 ppm by most of the extracts tested. However, at 100 ppm, only two extract *C. hierosolymitana* (L) and *E. gerardiana* (R) presented significant mortality rate (30 and 40%, respectively).

Antitumor potato disc assay

Average number of tumors at different concentrations of

the methanol extracts is as shown in Figure 1. Five of the seven extracts exhibited tumor inhibition at all the three concentrations tested. While *E. gerardiana* (R) and *C. leucanthemum* (A) showed significant inhibition at two concentrations tested, that is, 1000 and 100 ppm. Tumor inhibition was observed in concentration dependant mode. Extract of aerial parts of *Q. dilatata* presented highest percentage of tumor inhibition at all concentrations (Table 3). Statistical analysis by using ANOVA showed highly significant effect of concentration and extract. Effect of extracts on viability of *A. tumefaciens* was evaluated by using agar well diffusion method. No effect on viability of *A. tumefaciens* was observed in the case of the six extracts. However, *Q. dilatata* (A) were effective against *A. tumefaciens* and showed significant antibacterial activity (10.3 mm zone at 1000 ppm).

Radish seed phytotoxicity assay

Effect of four different concentrations (10,000, 1000, 100, and 10 ppm) of methanol extracts was studied on root growth inhibition or stimulation of radish seedling. All extracts inhibited root growth at 10,000 ppm. However two of the seven extracts, that is, *C. hierosolymitana* (R) and *Q. dilatata* (A) had growth stimulation effect at 100 and 10 ppm concentrations (Table 4 and Figure 2). Effect of concentrations and extracts remained significant ($P < 0.05$).

In the second experiment, the effect of two different concentrations of each extract (7500 and 1000 ppm) on seed germination was studied as a function of incubation period of seeds. A gradual increase in seed germination for all extracts was observed till the 5th day of incubation. Inhibition of seed germination was observed in the case of all extracts at 7500 and 1000 ppm (Figure 3). Effect of extracts as well as concentration and incubation period was significant ($P < 0.05$) with maximum inhibition by *E. gerardiana* (R) and *C. leucanthemum* (A) at 7500 ppm.

Phytochemical analysis

Phytochemical analysis showed the presence of different

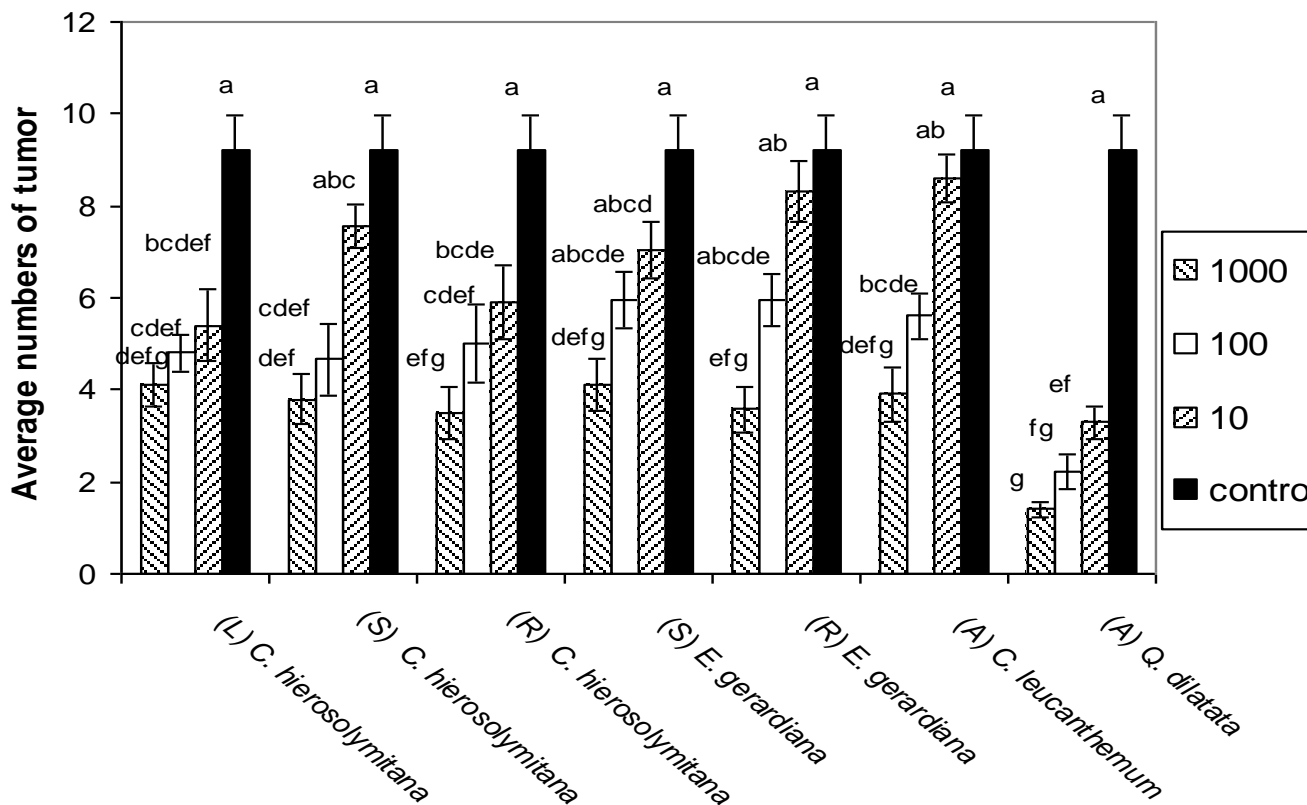


Figure 1. Average number of tumors at different concentrations of extracts. Values with similar letters are not significantly different from each other at P > 0.05.

Table 3. Percentage inhibition of tumor formation at different concentrations of the extracts.

Methanol extract	Tumor inhibition at concentrations (%)		
	1000 ppm	100 ppm	10 ppm
<i>C. hierosolymitana</i> (L)	55.43	47.83	41.30
<i>C. hierosolymitana</i> (S)	58.82	49.41	17.65
<i>C. hierosolymitana</i> (R)	61.96	45.65	35.87
<i>E. gerardiana</i> (S)	55.29	35.29	23.53
<i>E. gerardiana</i> (R)	61.18	35.29	9.41
<i>C. leucanthemum</i> (A)	57.61	39.13	6.52
<i>Q. dilatata</i> (A)	84.78	76.1	64.1

Table 4. Percentage root growth inhibition or stimulation at different concentrations of the extracts.

Methanol extract	Root growth regulation at concentrations (%)			
	10000 ppm	1000 ppm	100 ppm	10 ppm
<i>C. heirosolymitana</i> (L)	64.31	13.02	7.61	2.06
<i>C. heirosolymitana</i> (S)	56.93	13.13	2.80	1.75
<i>C. heirosolymitana</i> (R)	53.49	4.01	-3.93	-8.60
<i>E. gerardiana</i> (S)	84.24	15.83	9.93	8.11
<i>E. gerardiana</i> (R)	28.84	9.72	7.91	3.69
<i>C. leucanthemum</i> (A)	54.82	44.60	34.34	20.82
<i>Q. dilatata</i> (A)	21.99	18.54	-0.8	-3.54

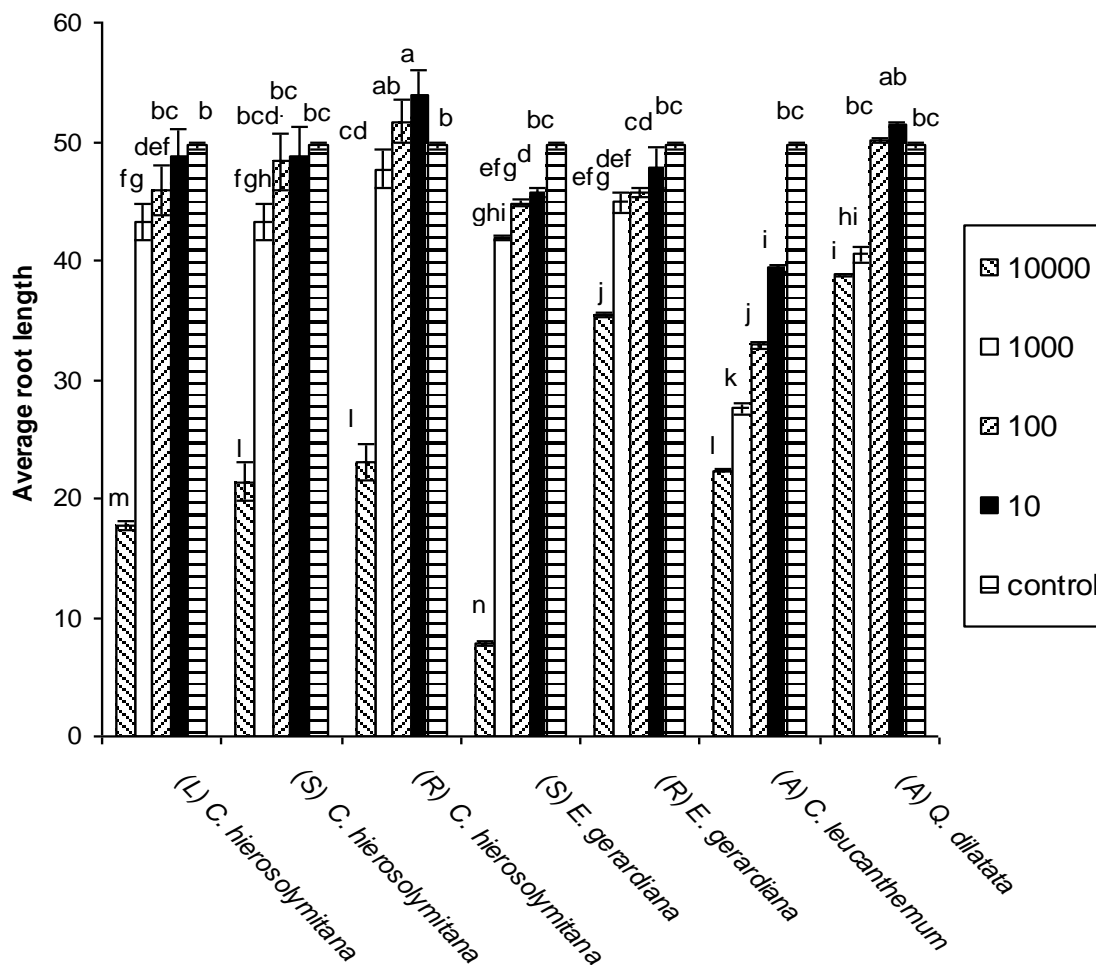


Figure 2. Effect of different concentrations (10,000, 1000, 100, and 10 ppm) on root length on 5th day. Values with similar letters do not show significant difference $P > 0.05$.

classes of the compound in different extracts. Results are shown in Table 5. Results showed that alkaloids were present in six of the seven extracts, only leaf extract of *C. hierosolymitana* had not shown positive results for alkaloids. Saponins were present in all the extracts at varying level. Five extracts (leaf, stem, and root extracts of *C. hierosolymitana*; stem extract of *E. gerardiana*; and aerial extract of *Q. dilatata*) were weakly positive, while two (root extract of *E. gerardiana* and aerial extract of *C. leucanthemum*) were moderately positive for saponins. Anthraquinones were present in four plant extracts, that is, leaf, stem, and root extracts of *C. hierosolymitana* and aerial extract of *Q. dilatata*. Coumarins were not detected in any of the plant extract. Terpenoids were present in four plant extracts (leaf extract of *C. hierosolymitana*, stem extract of *E. gerardiana*, aerial extracts of *C. leucanthemum*, and *Q. dilatata*). Flavonoids and flavones were found in six of the seven extracts; only stem extract of *C. hierosolymitana* had not shown positive results. Tannins were only present in four extracts, that is, leaf extract of *C. hierosolymitana*, aerial extract of *Q. dilatata*

(strongly positive), root extract of *C. hierosolymitana*, and aerial extract of *C. leucanthemum* (weakly positive). Phlobatannins were present in leaf, stem, and root extracts of *C. hierosolymitana*. Cardiac glycosides were present in stem extract of *C. hierosolymitana*, stem extract of *E. gerardiana*, and aerial extract of *Q. dilatata*. These compounds are known to have pharmacological activities and therefore are commonly found in medicinal plants.

DISCUSSION

Medicinal plant species all over the world have been playing a vital role in drug discovery efforts. For many plants, there is no relevant literature available; so, biological activity must be evaluated using more direct methods such as pharmacological testing or screening. Overall, extracts must be active in at least one of the bioassays adopted in this screening to be used for further studies.

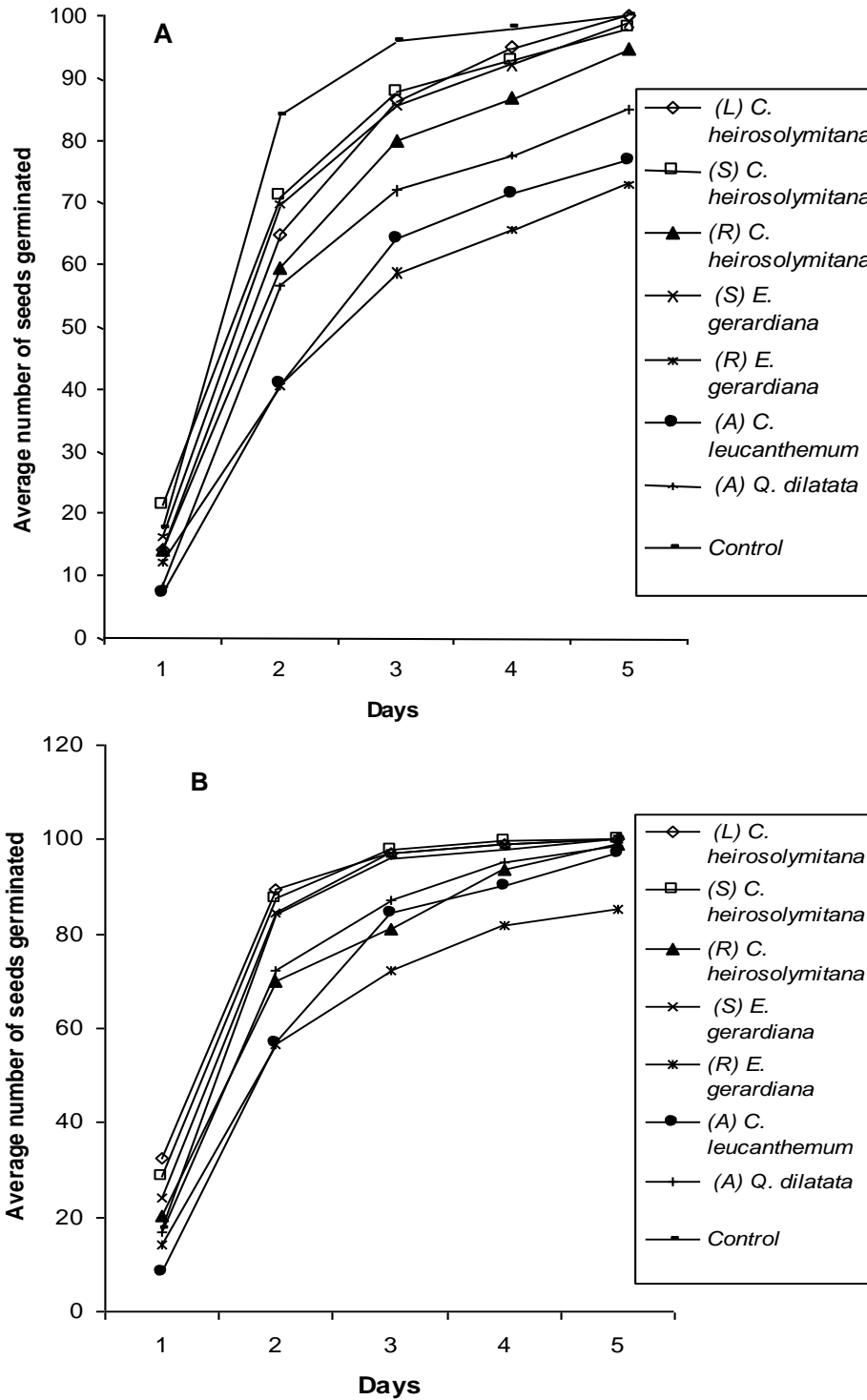


Figure 3. Effect of methanol extracts on seed germination as a function of incubation period of seeds at (A) 7500 ppm and (B) 1000 ppm.

Beneficial biological activity may be indicated from selected bioassay results; however, it is important to know the level of general toxicity of plant extracts as well.

The brine shrimp assay can be used to screen the plant extracts for their general toxicity and may help in discovery of suitable drug for cancer cells (McLaughlin et

Table 5. Phytochemical analysis of extracts.

Class of compounds	<i>C. hierosolymitana</i> (L)	<i>C. hierosolymitana</i> (S)	<i>C. hierosolymitana</i> (R)	<i>E. gerardiana</i> (S)	<i>E. gerardiana</i> (R)	<i>C. leucanthemum</i> (A)	<i>Q. dilatata</i> (A)
Alkaloids	Mayer's reagent	-	-	+++	+++	+++	+++
	Dragendorff's reagent	-	++	+++	-	+++	+++
Saponins	+	+	+	+	++	++	+
Anthraquinones	+	+	+	-	-	-	+
Coumarins	-	-	-	-	-	-	-
Terpenoids	Blue ring in green colour	-	-	Blue ring in green colour	-	Blue ring in green colour	Blue ring in green colour
Flavonoids, flavones,	Yellow	-	yellow	Yellow	Yellow	Yellow	Yellow
Tannins	+++	-	++	-	-	++	+++
Phlobatannins	Red ppt.	Red ppt.	Red ppt.	-	-	-	-
Cardiac glycosides	-	Green blue colour	-	Green blue colour	-	-	Green blue colour

+++ = Strongly positive; ++ = moderately positive; + = weakly positive; - = negative; Yellow = flavonoids, flavones positive; Blue ring in green colour = terpenoids positive; Red precipitate = phlobatannins positive; Green blue colour = cardiac glycosides positive.

al., 1991). Two of the seven extracts presented significant cytotoxicity. Highest rate of lethality to brine shrimp was observed in the case of leaf extract (*C. hierosolymitana*). A number of previous studies indicated potent cytotoxicity in the case of methanol extracts of several plant species, as Quignard et al. (2003) identified several cytotoxic plants when 226 methanolic and water extracts were screened for cytotoxicity towards brine shrimp larvae.

Antitumor potato disc assay is an acceptable tool to primarily screen antitumor activity of various crude extracts as well as purified fractions, or synthetic compounds regardless of the mode of inhibitory action on tumor formation (Ahmad et al., 2007, 2008; Hussain et al., 2007; Inayatullah et al., 2007). All methanol extracts tested exhibited moderate to high rate of inhibition of tumor formation at all tested concentrations, except *E. gerardiana* (R) and *C. leucanthemum* (A) which were not very effective at 10 ppm concentration (Table 3). *Q. dilatata* (A) presented significant inhibition of tumor formation, but this extract affected the viability of *A. tumefaciens* strain as well. Therefore, in this case, tumor

inhibition could partly or completely be because of antibacterial activity of the extract against *A. tumefaciens*. Previously, Galsky et al. (1980) examined the effects of several compounds and plant extract on crown gall tumor formation and found no effect on bacterial viability.

Radish seeds have been used in general toxicity studies, because of their sensitivity to phytotoxic compounds (Einhellig and Rasmussens, 1978) and are a standard assay in allelopathic studies (Patterson, 1986). All extracts in this study exhibited toxicity in radish seed bioassay at 10,000 and 1000 ppm indicating allelopathic potential of these plants. A very interesting feature of this study is the growth stimulation effect of two extracts, that is, *C. hierosolymitana* (R) and *Q. dilatata* (A) at low concentrations. Our results clearly indicate the presence of chemical factors in *C. hierosolymitana* (R) and *Q. dilatata* (A) which have growth regulatory effects against other plants, such as radish. In a previous study too, such plant extracts have been identified which showed growth enhancement at lower concentration, while growth inhibition effect at higher

concentration (Tsao et al., 2002).

In this study, phytochemical analysis showed that alkaloids, saponins, anthraquinones, flavonoids, flavones, hydrolysable tannins, phlobatannins (condensed tannins), terpenoids, and cardiac glycosides were present in *C. hierosolymitana*, *C. leucanthemum*, and *E. gerardiana* extracts. These results supported the findings of Abdel-Sattar (1985) and Hashim et al. (1990) that alkaloid ricinine and flavonoids were commonly found in genus *Chrozophora*. Alkaloids, saponins, terpenoids, flavonoids, flavones, and hydrolysable tannins were present in *C. leucanthemum*. Previously, sesquiterpene lactones (Haruna et al., 1981), flavonoids (Wilkomirski and Dubielecka, 1996), flavone and flavonone (Hu et al., 1994) have been isolated from *Chrysanthemum* plants. These plant extracts showed significant brine shrimp cytotoxic, antitumor, and phytotoxic activities. The presence of alkaloids in *E. gerardiana* was reported by Bown (1995). Ephedrine, the active alkaloid, tannins, saponin, and flavones were reported earlier in *Ephedra* plants (Chevallier, 1996). Alkaloids, saponins, anthraquinones, terpenoids,

flavonoids, flavones, hydrolysable tannins, and cardiac glycosides were present in *Q. dilatata*. This plant extract showed significant antitumor at all the three concentrations tested, while they showed growth stimulation at lower concentrations. The presence of terpenoids in *Q. dilatata* has also been reported by Evans (2002). In another study, hydrolysable tannins were isolated from *Quercus* spp. (Nishimura et al., 1984). Therefore, the present investigation is in accordance with other reports as Mojab et al. (2003) studied fifty five Iranian plants and found alkaloids in 39 plants, while flavonoids, tannins, and saponins were reported in 37, 20, and 44 plants, respectively. Hadi and Bremner (2001) studied 100 plant species for alkaloid and found 23% positive for alkaloids. The phytochemicals detected in our extracts are well known for various pharmacological activities. For example, alkaloids are common antibacterial, antimalarial, cytotoxic, and anticancerous agents (Wirasathien et al., 2006). Similarly, saponins have the insecticidal, antibiotic, fungicidal properties (Sparg et al., 2004). Anthraquinones are antibacterial, antifungal, and cytotoxic agents, while terpenoids are antimalarial and antibacterial agents (Kanokmedhakul et al., 2005). Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antineoplastic, antiviral, anti-thrombotic antioxidant, and vasodilatory activities (Miller, 1996). Tannins have shown potential antiviral (Lin et al., 2004), antibacterial (Akiyama et al., 2001), and antioxidant activities (Yokozawa et al., 1998). In the past few years, tannins have also been studied for their potential effects against cancer through different mechanisms (Bhagavathi et al., 1999). Cardiac glycosides have the cytotoxic properties and the Na⁺/K⁺-ATPase inhibitory properties (Joseph et al., 2005).

These compounds are known to have pharmacological activities and therefore are commonly found in medicinal plants.

Conclusion

Four simple bench top bioassays have been used which are useful for the screening of plant extracts for pharmacological activities. Crude methanol extracts screened in the present study can be analyzed further for discovery of important chemotherapeutic agents. Furthermore, extracts inhibiting tumor formation in antitumor potato disc assay can lead to discovery of new anticancerous drugs in future. Extracts with significant antitumor as well as antibacterial activity against *A. tumefaciens* can be used in treating fruit plants infected with crown gall disease. Some plant extracts showed plant growth regulatory effect against radish seeds by inhibiting the growth of root at higher concentrations and enhancing the growth at lower concentrations. Such extracts could lead to discovery of new natural herbicide and plant growth regulators. Further investigations are needed to isolate the individual chemicals possessing

these activities.

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