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EX-VIVO ANTIBACTERIAL, PHYTOTOXIC AND CYTOTOXIC, POTENTIAL IN THE CRUDE NATURAL PHYTOCONSTITUENTS OF *RUMEX HASTATUS* D. DON

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Abstract

In the present study the crude saponins, crude methanolic extract and subsequent fractions of *Rumex hastatus* have been evaluated for the cytotoxic potential against brine shrimps and phytotoxic potential against radish. In addition, the antibacterial activity has been evaluated for crude flavonoids (Rh.Fl), saponins (Rh.Sp), crude methanolic extract (Rh.Cr) and resultant fractions of *R. hastatus* by well diffusion method. In antibacterial assay, the Rh.Fl displayed considerably better activity against all the strains followed by Rh.Sp, which was comparable with positive control. Among the fractions the ethyl acetate (Rh.EaF) and chloroform fractions (Rh.CfF) revealed good zones of inhibition (ZOI). All the test samples were active against *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The largest ZOI was revealed by Rh.Fl i.e., 34.8 ± 0.72 mm ZOI against *Bacillus cereus*. The Rh.Sp was more active against *Escherichia coli* resulting in 34.1 ± 0.49mm ZOI. Similarly against brine shrimps, the Rh.Sp fractions show excellent cytotoxic i.e., 96.76 ± 1.1, 73.86 ± 2.9 and 50.67 ± 1.9% brine shrimps lethality at the concentration of 1000, 100 and 10µg/ml, respectively with the LC_{50} of 10µg/ml. Among the fractions, Rh.CfF showed remarkable cytotoxicity with LC_{50} of 65µg/ml. Rh.EaF and Rh.Cr displayed the similar lethality with LC_{50} of 90µg/ml. The cytotoxicity exhibited by aqueous fraction (Rh.Wtf) and n-hexane fraction (Rh.HxF) was moderate with LC_{50} of 100µg/ml and 390µg/ml, respectively. The phytotoxicity assay was conducted against radish seeds in which the Rh.Sp exhibited remarkable phytotoxicity i.e., root length inhibition (RLI) (IC_{50}= 9µg/ml) and seeds germination inhibitions (SGI) (IC_{50}= 15µg/ml) which was comparable with the Paraquat act as positive control. Among the fractions Rh.CfF demonstrated outstanding phytotoxic activity i.e., 86.63 ± 0.80, 67.10 ± 1.10 and 45.90 ± 2.90% (IC_{50} = 23 µg/ml) RLI and 78.68 ± 1.33, 69.92 ± 0.50 and 54.70 ± 1.20% (IC_{50} = 5µg/ml) SGI at the concentration of 1000, 100 and 10 µg/ml respectively. Rh.EaF demonstrated 85.42 ± 0.20, 63.00 ± 1.90 and 41.33 ± 1.10% (IC_{50} = 32µg/ml) RLI and 72.00 ± 2.32, 63.76 ± 1.10 and 49.70 ± 1.10% (IC_{50} = 13µg/ml) SGI at 1000, 100 and 10 µg/ml respectively. The Rh.HxF revealed 82.59 ± 3.20, 61.33 ± 1.90 and 39.33 ± 1.10% (IC_{50} = 35µg/ml) RLI and 68.00 ± 2.32, 57.53 ± 0.70 and 36.20 ± 0.90% (IC_{50} = 46 µg/ml) SGI at 1000, 100 and 10 µg/ml respectively. The Rh.Cr and Rh.WtF showed moderate phytotoxicity. Based on the results obtained from the present study it may be inferred that *R. hastatus* may be an excellent source of natural compounds having antibacterial, anticancer and herbicidal potential.

Key words: *Rumex hastatus*, Cytotoxicity, Phytotoxicity, Antibacterial activity, Saponins, Flavonoids

Introduction

From the very start, medicinal plants have been contributing a lot in the primary health care worldwide. Natural products are the major source of clinical medicine since long. Plethora of research on medicinal plants has explored the treatment of several challenging diseases (Plummer *et al.*, 2001). One of the challenging diseases is neoplasia which is under consideration of researchers (Tonack *et al.*, 2009). Each and every disease is having a specific origin and the origins of majority of diseases are microbes. Antibiotics are the drugs which are used to combat various diseases of microbial origin. But the main problems with the antibiotics are adverse drug reactions and microbial resistance (Levy, 1982; Iwata & Akita, 1997).

The remedies of challenging diseases are veiled in the medicinal plants which are authenticated from time to time (Kumar *et al.*, 2007). Several plant extracts have been reported to possess strong antimicrobial activities (Nitta *et al.*, 2002; Walter *et al.*, 2011). The ethnobotanical use of plants provides a glimpse for the scientific verification and isolation of valuable compounds from plants for possible cure of specific diseases. Whole population of the world depends upon the plant's products in so many ways i.e., vegetables, fruits, grains, timber, furniture, medicines etc are obtained from the plants which are the basic needs of humans (Della Penna, 1999; Fabricant & Farnsworth, 2001; Ibrar *et al.*, 2007). The main focus of the world is to grow more and more plants and to increase the agricultural outcome but unfortunately there are so many factors, which are responsible for the downfall of agriculture. The most prominent factors are pests and weeds (Zehnder *et al.*, 2007; Jan *et al.*, 2010). Various pesticides including herbicides are employed for the control of these factors. The synthetic herbicides used against weeds are definitely efficient but they have several long term hazardous and adverse effects (Yousef *et al.*, 1995; Azmi *et al.*, 2006; Pakulska & Czerczak, 2006). The natural herbicides obtained from plant materials are having negligible adverse effects (Vyvyan, 2002).

R. hastatus D. Don belongs to the family polygonaceae and it is distributed worldwide which are extensively used ethnobotanically for different purposes (Rao *et al.*, 2011). Numerous pharmacological activities have been performed on various species of this genus. *R. hastatus* having a lot of folklore uses, especially it is used in GIT ailments, cuts, wounds, bleedings, as appetizer, as anthelmintic, in snake bites etc (Bhatt & Negi, 2006; Ali

& Qaiser, 2009; Rokaya *et al*., 2010). The saponins obtained from various plants are verified to have antioxidant, anticancer, anthelmintic and insecticidal properties (Lacaille-Dubois & Wagner, 1996; Xi *et al*., 2008; Yan *et al*., 2009; Wang *et al*., 2010; Ali *et al*., 2011). Based on the literature survey and ethnomedicinal uses of *R. hastatus*, the Rh.Sp, Rh.Cr and subsequent fractions of this plant are evaluated for its possible antibacterial, cytotoxic and phytotoxic potentials in the current study.

Materials and Methods

Extraction: The whole plant of *R. hastatus* was collected in April from district Dir (Lower) of Malakand division, Khyber Pakhtunkhwa (KPK), Pakistan. The plant voucher specimen 1015 SA was identified by Dr. Ali Hazrat, Plant Taxonomist, Department of Botany, Shaheed Benazir Bhutto University, Sheringal Dir Upper, KPK, Pakistan. The plant was subjected to shade drying and crude methanolic extraction through the method used by (Kamal *et al*., 2015 & Ayaz *et al*., 2014). The crude methanolic extract (Rh.Cr) obtained was 400g.

Fractionation: Crude methanolic extract weighing 300g was suspended in 500 ml of distilled water and fractionated through successive solvent-solvent extraction method starting from less polar solvent towards high polar solvents (Owoyele *et al*., 2008). The fractions obtained were *n*-hexane, chloroform, ethyl acetate and aqueous weighing 19, 21, 29 and 120g respectively.

Extraction of crude saponins: For extraction of crude saponins from *R. hastatus*, 20g plant powder was put in a conical flask and 100 ml of 20% ethanol was added to the conical flask. The sample was heated at 55°C in the water bath for four hours with continuous stirring. After four hours, the sample obtained was filtered and the residue having greenish color was re-extracted with 200ml of 20% ethanol. The sample after extraction was heated until a concentrated volume of 40ml was obtained. The sample obtained was transferred into a separating funnel and 20 ml of diethyl ether was added to it. After vigorous shaking, the separating funnel was put in a stand to get two layered sample. The lower layer was collected, which was aqueous layer while the upper diethyl ether layer was discarded. The aqueous layer obtained was diluted with 60 ml of *n*-butanol and the combined *n*-butanol extract was washed with 10 ml of 5% sodium chloride solution. The final solution obtained was kept in a hot water bath until complete evaporation and the Saponins obtained were dried in an oven yielding 1.3 g of crude Saponins (Khan *et al*., 2011).

Extraction of Flavonoids: For the extraction of Flavonoids, the procedure of Harborne was followed (Harborne, 1998). Plant sample weighing 5g was taken and boiled in 50ml of 2M HCl under reflux for half an hour. It was cooled and filtered using whatman No.42 filter paper. The extract was treated with equal volume of ethyl acetate. The flavonoids present in the extract were precipitated which were recovered with the help of weighed filter paper.

Antibacterial activity: Eight bacterial strains i.e., *Proteus mirabilis* (13315), *Staphylococcus aureus* (29213), *Escherichia coli* (739), *Bacillus cereus*, *Salmonella typhi* (locally identified), *Klebsiella pneumoniae* (700603), *Pseudomonas aeruginosa* (27853) and *Enterococcus faecalis* (29212) were used to assess antibacterial activity of plant samples which were acquired from Microbiology laboratory of Department of Microbiology, Quaid-e-Azam University Islamabad. These were sub-cultured on sterile nutrient agar media.

Preparation of inocula: A sterile wire loop was used for the preparation of inocula. A wire loop filled with bacterial culture was dipped in sterile water for the preparation of inocula.

Procedure: Wells of 5mm diameter were formed in sterilized and solidified nutrient agar media, using sterile cork borer. Sterile swab was used to inoculate the petri plates with bacterial cultures. Each petri plate was labeled with the name of specific inoculum. Samples having volume of 100 µl were added into the wells using a micropipette. Ceftriaxone was used as positive control, which was added into the central well of each petri plate. After inoculation and addition of samples, the petri plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) of each sample was measured (Mufti *et al*., 2012). Each sample was used in triplicate and data were represented as mean ± SEM.

Phytotoxicity (radish seed) assay: In the phytotoxicity assay the procedure reported by Arzu and Camper (2002) was followed (Turker & Camper, 2002). Plant samples weighing 20 mg were dissolved in 2 ml of methanol to make stock solution. 5, 50 and 500 µl of samples were transferred into separate petri plates from each stock solution to get the concentration of 10, 100 and 1000 µg/ml. In each petri plate, a sterilized filter paper was kept and the solvent was allowed to evaporate from plates. After complete evaporation of solvent, 5 ml distilled water was added to each petri plate. The petri plate having sterilized filter paper and 5 ml distilled water served as control. To each petri plate 25 radish seeds which were previously sterilized with 0.1% mercuric chloride were added and incubated at room temperature for five days. On the fifth day the plates were examined for the number of seeds germinated and their root length were measured. The whole procedure was conducted in triplicate and the data obtained were recorded as mean ± SEM.

Brine shrimp Cytotoxicity: The procedure of Meyer *et al*., (2007) was followed for the cytotoxicity assay of *R. hastatus*. Requirements for the brine shrimp cytotoxicity assay are eggs of *Artemia salina* (shrimps eggs), sample solutions, simulated sea water (38g per liter of distilled water having pH of 7.4), tray for hatching having perforated partition, torch for attracting shrimps larvae, vials for samples, micropippete (for sucking 5, 50 and 500 microlitres of samples), D/water, standard drug (Etoposide) and methanol.

Hatching techniques: Brine solution was prepared, filtered and a hatching tray was half filled with this solution. 50mg of eggs of shrimps were sprinkled into one partition of hatching tray and kept at 37°C in the incubator for hatching for 24 hours. After hatching the larvae were kept under illumination for maturation for next 24 hours. After maturation the different plant samples were applied on the shrimps nauplii to evaluate the cytotoxic effect.

Procedure: Test samples having the concentrations of 10, 100, 500 and 1000µg/ml were prepared by dissolving 20mg of the extracts in 2 ml of DMSO and transferring 5, 50 and 500µl of the test samples respectively into the vials. The vials were kept for a while to evaporate the solvent. Each concentration was made in triplicate. To each vial 30 shrimps nauplii were transferred using a Pasteur pipette and the volume of each vial was made 5 ml using simulated sea water. For negative control 30 shrimps nauplii were transferred into vial and making the volume 5 ml using simulated sea water. The reference cytotoxic drug (Etoposide) was used as positive control. The vials were kept at room temperature for 24 hr. and the mortality was evaluated by counting the number of killed shrimps in each vial. For percentage mortality the following equation was used,

$$\% \text{ mortality} = \frac{(\text{number of dead shrimps nauplii}}{\text{total number of shrimps nauplii)}} \times 100$$

Statistical analysis: One-way ANOVA followed by Dunnett's multiple comparison tests were applied for the comparison of positive control with the test groups using Graph Pad Prism. *P* values less than or equal to 0.05 were considered statistically significant. Microsoft Excel software was used to calculate LC_{50} and IC_{50} values. The standard error of mean (SEM) were calculated at 95% confidence intervals using Graph Pad Prism.

Results

Antibacterial effect: The antibacterial activity of different samples of *R. hastatus* is shown in Table 1. In this assay, crude flavonoids displayed considerably better activity against all the strains followed by saponins. The flavonoids showed marvelous activity against all the strains which was comparable with the standard. Among the fractions the ethyl acetate and chloroform fractions revealed good performance. All the test samples were active against *Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The aqueous and ethyl acetate fraction showed no activity against *Enterococcus faecalis*. The methanolic and aqueous fractions were inactive against *Escherichia coli* while the chloroform and saponins were inactive against *Salmonella typhi.* The largest zone of inhibition was revealed by Ceftriaxone i.e., 35.3±0.36 mm against *Proteus mirabilis* followed by the crude flavonoids showing 34.8±0.72 mm zone of inhibitions against *Bacillus cereus*. Saponins were more active against *Escherichia coli* resulting in 34.1±0.49 mm zone of inhibitions. All the samples were approximately least active against *Salmonella typhi* except the methanolic fractions, which resulted in 23.1±0.43 mm zone of inhibitions against this strain. From the above results it may be concluded that all the tested samples of *R. hastatus* are rich in antibacterial components especially the flavonoids, saponins, ethyl acetate and chloroform fractions.

Table 1. Zones of inhibition of various samples against bacterial strains.

Samples	Pm	Sa	Ec	Bc	St	Kp	Pa	Ef
Rh.Cr	9.9±0.40	8.7±0.51	na	8.7±0.26	23.1±0.43	12.4±0.84	7.4±0.83	22.7±1.84
Rh.HxF	16.4±0.41	7.9±0.75	8.3±0.37	8.0±0.53	8.5±0.82	22.6±0.35	11.3±0.69	19.8±1.18
Rh.CfF	12.2±0.33	8.9±0.75	17.4±0.68	31.3±0.44	na	12.2±0.66	7.4±0.66	31.9±0.60
Rh.EaF	11.5±1.17	10.9±0.26	22.7±0.23	7.7±0.45	18.1±0.43	14.4±0.81	16.1±0.48	na
Rh.WtF	10.3±0.43	13.2±0.67	na	12.0±0.37	7.3±0.75	18.9±0.81	8.3±1.08	na
Rh.Sp	9.5±0.92	16.6±0.50	34.1±0.49	24.5±0.86	na	11±0.51	16.8±0.96	19.3±0.65
Rh.Fl	13.1±0.24	11.3±0.72	17.2±0.40	34.8±0.72	7.3±0.70	33.6±0.47	15.5±0.73	15.5±0.68
CEF	35.3±0.36	32.3±0.65	9.1±0.52	na	19±0.37	21±0.51	26.1±0.55	17.1±1.12

Data is represented as mean±SEM, (n=3).

Keys: *Pm*: *P.mirabilis, Sa: S.aureus, Ec: E.coli, Bc: B.cereus, St: S.typhi, Kp: K.pneumonia, Pa: P.aeruginosa, Ef: E.faecalis,* Rh.Cr: Crude methanolic extract; Rh.HxF: *n*-hexane fraction; Rh.CfF: Chloroform fraction; Rh.EaF: Ethyl acetate fraction; Rh.WtF: Aqueous fraction; Rh.Sp: Saponins; Rh.Fl: Flavonoids; CEF: Ceftriaxone, na: no activity.

Effect of phytotoxicity: To evaluate the allelopathic potential of plant extract, the radish seed germination assay was conducted. The number of seeds germinated and the average root length were figured out. Saponins exhibited remarkable phytotoxicity i.e., root length inhibition (IC_{50} = 9 µg/ml) and seed germination inhibition (IC_{50} = 15 µg/ml) which was comparable with the positive control (Paraquat) having root length inhibition and seed germination inhibition (IC_{50} = <1 µg/ml). The chloroform and ethyl acetate fraction showed outstanding phytotoxic effect i.e., percent root length inhibition shown by chloroform was 86.63 ± 0.80, 67.10 ± 1.10 and 45.90 ± 2.90 (IC_{50} = 23µg/ml) at the concentration of 1000, 100 and 10 µg/ml respectively

while the seed germination inhibition was 78.68 ± 1.33, 69.92 ± 0.50 and 54.70 ± 1.20 (IC_{50} = 5µg/ml) at 1000, 100 and 10 µg/ml respectively. The ethyl acetate fractions demonstrated 85.42 ± 0.20, 63.00 ± 1.90 and 41.33 ± 1.10% (IC_{50} = 32µg/ml) root length inhibitions and 72.00 ± 2.32, 63.76 ± 1.10 and 49.70 ± 1.10% (IC_{50} = 13 µg/ml) seed germination inhibition at 1000, 100 and 10 µg/ml respectively. The *n*-hexane fractions revealed 82.59 ± 3.20, 61.33 ± 1.90 and 39.33 ± 1.10% (IC_{50} = 35µg/ml) root length inhibition and 68.00 ± 2.32, 57.53 ± 0.70 and 36.20 ± 0.90% (IC_{50} = 46 µg/ml) seed germination inhibition at 1000, 100 and 10 µg/ml, respectively. The methanolic and aqueous fractions showed moderate phytotoxicity i.e., the methanolic fraction display 72.67±5.67, 56.76±1.90 and 33.56±1.10% (IC_{50} = 49 µg/ml) root length inhibition and 70.68±3.52, 46.53±0.90 and 39.90±1.10% (IC_{50} = 240 µg/ml) seeds germination inhibitions at 1000, 100 and 10 µg/ml respectively as shown in Table 2. The aqueous fraction exhibited 68.01 ± 0.20, 49.23 ± 2.90 and 28.00 ± 1.90% (IC_{50} = 118 µg/ml) root length inhibition and 58.64 ± 3.52, 42.46 ± 0.50 and 23.53 ± 0.70% (IC_{50} = 502 µg/ml) seeds germination inhibition at 1000, 100 and 10 µg/ml respectively. The overall results of radish seeds phytotoxicity assay made obvious, that *R. hastatus* is having strong phytotoxic potential. It may be concluded that the plant species which are grown beside the *R. hastatus* may be affected by the leaves which fall from this plant. As reported by Inderjit (1996), the major class which have allelopathic effect are the phenolics and phytochemical screening of *Rumex* genus has been reported by Fatima et al (2009) who revealed different types of polyphenols which may be responsible for the phytotoxic effect (Inderjit, 1996; Fatima *et al.*, 2009).

Table 2. Concentration dependent Phytotoxic effect of Rh.Cr, Rh.HxF, Rh.CfF, Rh.EaF and Rh.WtF of *R.hastatus* against radish seeds.

S.No	Samples	Conc. (µg/ml)	Root length inhibition % (mean±SEM)	IC_{50} (µg/ml)	Germination inhibition% (mean±SEM)	IC_{50} (µg/ml)
01	Rh.Cr	1000	72.67±5.67***		70.68±3.52***	
		100	56.76±1.90***	49	46.53±0.90***	240
		10	33.56±1.10***		39.90±1.10***	
02	Rh.HxF	1000	82.59±3.20***		68.00±2.32***	
		100	61.33±1.90***	35	57.53±0.70***	46
		10	39.33±1.10***		36.20±0.90***	
03	Rh.CfF	1000	86.63±0.80***		78.68±1.33***	
		100	67.10±1.10***	23	69.92±0.50***	5
		10	45.90±2.90***		54.70±1.20***	
04	Rh.EaF	1000	85.42±0.20***		72.00±2.32***	
		100	63.00±1.90***	32	63.76±1.10***	13
		10	41.33±1.10***		49.70±1.10***	
05	Rh.WtF	1000	68.01±0.20***		58.64±3.52***	
		100	49.23±2.90***	118	42.46±0.50***	502
		10	28.00±1.90***		23.53±0.70***	
06	Rh.Sp	1000	85.33±0.49***		83.00±1.15***	
		100	73.67± 0.89***	9	68.73±0.78***	15
		10	51.80 ±1.60***		47.33±1.10***	
07	PC	1000	93.64 ± 1.60		98.61± 1.70	
		100	86.20 ±1.40	<1	83.22 ±0.23	<1
		10	79.33 ±1.20		76.93 ± 0.49	

Data is represented as mean±SEM, (n=3).

Key: Rh.Cr = Crude methanolic extract; Rh.HxF = *n*-hexane fraction; Rh.CfF = Chloroform fraction; Rh.EaF = Ethyl acetate fraction; Rh.WtF = Aqueous fraction, Rh.Sp = Saponins, PC = Positive control (Paraquat)

Effect of cytotoxicity: The cytotoxic effect of crude methanolic, *n*-hexane, chloroform, ethyl acetate, aqueous fractions and saponins were measured by evaluating the brine shrimps lethality in different concentrations as shown in the Table 3. The activity of each sample was dose dependent. The cytotoxic effect of crude saponins was outstanding in each concentration while the chloroform fraction excelled among all fractions in as shown in Table 3. The saponins showed 96.76 ± 1.1, 73.86 ± 2.9 and 50.67 ± 1.9% lethality at the concentration of 1000, 100 and 10µg/ml, respectively, having the LC_{50} of 10µg/ml which was comparable with the positive control (Etoposide LC_{50} = 9.8µg/ml). The chloroform fractions demonstrated 90.23 ± 2.9, 66.33 ± 1.8 and 26.26 ± 1.1% lethality at 1000, 100 and 10µg/ml respectively with LC_{50} of 65µg/ml. The crude methanolic fractions and ethyl acetate fractions showed the same cytotoxic potential i.e., both having the LC_{50} of 90µg/ml. Similarly the *n*-hexane and aqueous fractions showed moderate cytotoxic potential i.e., the *n*-hexane displayed 66.90 ± 2.9, 33.33 ± 1.8 and 16.86 ± 1.1% lethality and aqueous fractions exhibited 73.43 ± 1.1, 50.00 ± 1.9 and 33.56 ± 2.9% lethality at the concentration of 1000, 100 and 10µg/ml respectively. The LC_{50} determined for *n*-hexane and an aqueous fraction was 390 and 100µg/ml respectively.

Discussion

Evaluation of various samples of *R. hastatus* suggests that this plant possesses strong antibacterial potential. The Rh.Fl revealed promising results against various bacterial strains. This group of compounds has already been reported to have excellent anti-infective properties and numerous compounds of flavonoids having strong antibacterial properties have been indentified and characterized (Cushnie & Lamb, 2005). It has also been reported that several compounds of flavonoids in combination possess synergistic effect i.e., quercetin, isorhamnetin-3-rutinoside and rutin, present in the extract of *Marrubium globosum* have better antimicrobial potential as compared to the single compound (Kimura & Yamada, 1984). The saponins isolated from *R. hastatus* also resulted in significant antibacterial activity against various bacterial strains. The antimicrobial activity of saponins has also been reported by several researchers (Avato *et al.*, 2006). Moreover cytotoxicity test is the key to develop anticancer drugs and to isolate anticancer compounds from medicinal plants (Kerwin, 2004). The brine shrimp cytotoxicity assay was conducted in the current study for the determination of anticancer properties of this plant. From the results it is determined that the cytotoxicity order for *R. hastatus* is crude saponins > chloroform fractions > ethyl acetate fractions > methanolic fractions > *n*-hexane fractions > aqueous fractions. The brine shrimp lethality was highest in the saponins, so it may be concluded that the saponins may be responsible for the lethality of brine shrimps or saponins are the compounds having anticancer properties. The ethyl acetate fraction and chloroform fraction have shown the prominent cytotoxicity so it may be deduced that the saponins and other constituents responsible for the cytotoxicity are in large quantity in these fractions. As reported by Rehman *et al.,* (2009) the positive co-relation exist between the brine shrimp lethality assay and human nasopharyngeal carcinoma (KB cell line) (Mclaughlin *et al.*, 1998; Mannan *et al.*, 2009). So the *R. hastatus* may be a good source of natural anticancer products.

Radish seeds were used to carry out the phytotoxic activity of *R. hastatus*. For the phytotoxicity assay, the parameters employed were seed germination inhibitions and root length inhibitions. The phytotoxic effect was highest for crude saponins and chloroform fractions while lowest for the aqueous fractions as shown in the Table 3. It may be concluded from the data, that the quantity of constituents responsible for the phytotoxic effect is highest in the chloroform fractions, ethyl acetate fractions and methanolic fractions of *R. hastatus* so these fractions may be rich in the active constituents having weeds killing potential. Weeds play a major role in diminishing the agricultural outcome for many crops (Barberi, 2002). Various techniques are employed to eliminate the weeds from the crops in which the use of chemical herbicides are the most prominent one, but having high toxicity, cancer causing effect and promoting environmental pollution, the use of various herbicides is discouraged (Morrison *et al.*, 1992; Neuberger *et al.*, 1999; Alexander, 2000; Macedo *et al.*, 2008).

To avoid the use of hazardous herbicides, the chemical agents should be replaced by natural herbicides. This study encourages the isolation of active principles of *R. hastatus* to be used as a natural weed killer and to enhance the percent agricultural outcome.

Table 3. Concentration dependent cytotoxic effect of Rh.Sp, Rh.Cr, Rh.HxF, Rh.CfF, Rh.EaF and Rh.WtF of *R. hastatus* against *Artemia salina.*

Samples	Total treated	Dose (μg/ml)	Percent cytotoxicity (mean ± SEM)	LC_{50} (μg/ml)
Rh.Cr	30	1000	$83.26 \pm 1.1^{***}$	90
		100	$52.76 \pm 1.1^{***}$	
		10	$30.33 \pm 1.8^{***}$	
Rh.HxF	30	1000	$66.90 \pm 2.9^{***}$	390
		100	$33.33 \pm 1.8^{***}$	
		10	$16.86 \pm 1.1^{***}$	
Rh.CfF	30	1000	$90.23 \pm 2.9^{***}$	65
		100	$66.33 \pm 1.8^{***}$	
		10	$26.26 \pm 1.1^{***}$	
Rh.EaF	30	1000	$86.10 \pm 1.1^{***}$	90
		100	$53.43 \pm 2.9^{***}$	
		10	$30.76 \pm 1.1^{***}$	
Rh.WtF	30	1000	$73.43 \pm 1.1^{***}$	100
		100	$50.00 \pm 1.9^{***}$	
		10	$33.56 \pm 2.9^{***}$	
Rh.Sp	30	1000	96.76 ± 1.1^{ns}	10
		100	73.86 ± 2.9^{ns}	
		10	50.67 ± 1.9^{ns}	

Standard drug; etoposide, LD50 = 9.8μg/ml; Data is represented as mean±SEM, (n=3).

Key: Rh.Cr = Crude methanolic extract; Rh.HxF = *n*-hexane fraction; Rh.CfF = Chloroform fraction; Rh.EaF = Ethyl acetate fraction; Rh.WtF = Aqueous fraction; Rh.Sp = Saponins.

Conclusion

Based on the results obtained from antibacterial, cytotoxicity and phytotoxicity assay it is inferred that *R. hastatus* is having strong antimicrobial, antineoplastic and herbicidal potential. Being the immense need of safe and efficacious natural antibiotics, herbicides and anticancer drugs, the crude flavonoids, saponins and chloroform fractions of *R. hastatus* should be subjected to isolation, purification and characterization to get valuable antibiotics, anticancer and herbicidal compounds.

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