Research Article

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Phytochemical analysis, Antioxidant and Antiprotoscolices potential of ethanol extracts of selected plants species against *Echinococcus granulosus*: In-vitro study

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Abstract: Cystic Echinococcosis is a serious zoonotic parasitic infection caused by *Echinococcus granulosus* species complex. The current study was designed to evaluate the in-vitro antiprotoscolices effect of alcoholic extracts of three selected medicinal plants including *Buxus Wallichiana*, *Berberis vulgaris* and *Euphorbia helioscopia* against *Echinococcus granulosus*. Fertile hydatid cysts were collected from livestock and viability of the protoscolices was confirmed by 0.1% eosin red stain method. Protoscolices were subjected to three different concentrations of alcoholic extracts (10mg/ml, 30mg/ml and 50mg/ml) for 10, 20 and 30 min. The highest efficacy was shown by *B. vulgaris* (97.92%) followed by *B. wallichiana* (65.98%) and *E. helioscopia* (61.22%) respectively, after exposure of 30 minutes at 50mg/ml concentration, that lead to the significant reduction in the

viability of protoscolices. Alkaloids, flavonoids, tannins and saponins were identified qualitatively and weighted quantitatively, that might help in the identification of bioactive compounds involved in selective action on the tegument layer of protoscolices. Alcoholic extracts of all the three selected medicinal plants showed toxic activities against protoscolices of *Echinococcus granulosus*. These findings suggest that all the selected medicinal plants could be a promising source of potent antiprotoscolices effect. However, the mechanism by which plant extracts killed protoscolices and also their safety for living cells are unclear and need to be investigated further.

Keywords: Phytochemical Analysis, Alcoholic extracts, Medicinal Plants, protoscolices.

1 Introduction

Cystic Echinococcosis (CE) or Hydatidosis is a zoonotic parasitic disease, caused by the larval stage of small tapeworm of dog of genus *Echinococcus*. CE causes extensive mortality and morbidity in human and livestock [1]. CE listed as one of the neglected tropical disease by World Health Organization [2]. As a result of CE, Hydatid cysts developed in internal organs mainly in liver and lungs of intermediate hosts and also in humans as unilocular fluid filled bladder [3]. This infection remains asymptomatic initially for many years or even permanently [4]. Eventually, malfunction and even death may occur due to continuous growth of cysts, without efficient treatment [5]. Chemotherapy doesn't often work and may result in complications; therefore, in some situations surgery involvement becomes mandatory [6]. The use of efficient protoscolicidal agent is necessary in order to reduce the risks of intraoperative spillage of the cysts fillings during

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Table 1: Selected medicinal plants for antiprotoscolices effect against *E. granulosus*.

S. No	Botanical names	English name	Vernacular name	Family
1	<i>B. Wallichiana</i>	Boxwood	Shamshad	Buxaceae
2	<i>B. vulgaris</i>	Barberry	Zark	Berberidaceae
3	<i>E. heliscopia</i>	Sunspurge	Gandi booti	Euphorbiaceae

surgery and consecutively reoccurrence of CE in almost 10% of the postoperative cases [7]. A variety of protoscolicidal agents have been in practice to inactivate the contents of the cysts, however mostly are not safe because of their side effects including sclerosing cholangitis, liver necrosis and methemoglobinemia [8]. New drugs from those sources that are not yet explored will be one of the features to find new alternative to treat CE. To date, many protoscolicidal agents including some plant extracts such as *Zataria multiflora* [9], *Endophytic Pestalotiopsis sp.* [10], *Saturena khuzestanica* [11], *Thymus vulgaris* and *Salvia officinalis* [12], *Mentha piperita* [13], *Trachyspermum ammi* [14], *Allium sativum* [15], *Nigella sativa* [16], menthol, ABZ, chlorhexidine gluconate (Chx-Glu), honey, cetrimide, hypertonic saline, povidone-iodine, ethanol and H₂O₂ have been used for inactivation of the hydatid cyst content however may cause intolerable side effects [17] which may result in limiting their use. In last few decades, many medicinal plants and their products are reported in Pakistan that are important in the treatment of many parasitic infections but could not be advanced into viable drugs. *Buxus Wallichiana* was used as bitter tonic, diaphoretic, vermifuge, antihelmentic, antireumatic, analgesic, antiepileptic, antileprotic and in hemorrhoids [18]. All parts of *Berberis vulgaris* have been investigated for medicinal values including antimicrobial, antipyretic, antipruritic, antimitic and cholagogue actions, jaundice, dysentery, cholecystitis, leishmaniasis, gall stones and cholelithiasis [19]. *Euphorbia heliscopia* has also great medicinal importance, often used to treat edema, ascites, pulmonary tuberculosis, tinea and febrifuge, cathartic, antihelminthic and purgative [20]. However, no previous laboratory studies have been documented the protoscolicidal effect of *B. Wallichiana*, *B. vulgaris* and *E. heliscopia* on protoscolices of hydatid cysts. Therefore, this study is the first attempt to underline the *in-vitro* effect of these selected medicinal plants, collected from various regions of Khyber Pakhtunkhwa, province of Pakistan, against the viability of *E. granulosus* protoscolices and to analyze the extracts of selected medicinal plants qualitatively and quantitatively to identify major biologically active phyto-constituents. It is expected that these active residents will provide useful information in

determining new compounds with better potency against the protoscolices of *E. granulosus*.

2 Materials and Methods

2.1 Plant Material

The aerial parts of selected medicinal plants including *B. wallichiana*, *B. vulgaris* and *E. heliscopia* (Figure-1, a, b and c) were collected from different localities of province Khyber Pakhtunkhwa, Pakistan. These plants species were recognized by Dr Mushtaq Ahmad (plant taxonomist) (Qaid Azam University, Islamabad) and the local names and family names are given in Table-1. The Voucher specimens of *B. wallichiana* (BW1), *B. vulgaris* (BV-1) and *E. heliscopia* (EH-1) of the plants are deposited in the local herbarium of Abdul Wali Khan University Mardan Pakistan. The 500 g shade dried powdered aerial parts of each plant were soaked in ethanol separately for 24 hours, then ethanol was filtered and evaporated with the help of rotary vacuum evaporator (Ollital, Xiamen, China). The greenish dried residue left after evaporation was stored for making stock solution in refrigerator (Dawlance, Pakistan) [21].

2.2 Collection of Hydatid Cysts and determination of protoscolices viability

Hydatid cysts were collected from the liver of naturally infected livestock by visiting different abattoirs in various districts of Khyber Pakhtunkhwa, province of Pakistan. Each cyst washed many times with sterile PBS (pH: 7.2) (*Thermo Fisher Scientific*, USA) and surface was sterilized with 70% ethanol (Merck, Darmstadt, Germany) and their fertility was examined. Each cyst was incised or aspirated with proper care and hydatid cyst fluid (HCF) was evacuated containing protoscolices in 15 mL falcon tubes (*Thermo Fisher Scientific*, USA), completely. These were left to precipitate for 60 min, at room temperature to obtain hydatid sand. To maintain the protoscolices for

a) *Buxus Wallichiana* b) *Berberis vulgaris* c) *Euphorbia helioscopia*

Figure 1: a, b and c shows selected medicinal plants for antiprotoscolices potential against *E. granulosus*.

all experiments, sterile preservative solution made up of a mixture of Kreb-Ringer solution and hydatid cyst sand (4:1) was used and observed for the viability of protoscolices by amoeboid like peristaltic movement under microscope (Olympus, Japan) [22]. Confirmation of results was further accessed by being stained with eosin solution (0.1% aqueous) (Merck, Darmstadt, Germany). The protoscolices were classified as dead when they stained and viable when they did not stain [23]. Viable protoscolices in the sediment were considered to be appropriate for experiment. The viability rate was measured by counting 100 protoscolices minimum, as number of viable protoscolices to total protoscolices. When the samples have 95% or more viable

protoscolices was considered to be appropriate for further experimentation and transferred to the container having normal saline and stored at 4°C until used.

2.3 Determination of *in-vitro* assay

Stock Solutions of alcoholic extracts were made by dissolving 2.5 gram of crude extracts in 10 mL of distilled water. One gram of each extract was dissolved in 9.9 mL of saline solution for making further dilutions. Moreover, dimethyl sulphoxide (DMSO) was added to increase the dispersal of each extract in normal saline and subsequently serial dilutions were prepared to obtain extracts at 10, 30 and 50 mg/mL. The *in-vitro* assay was conducted in sets of tubes as described previously [10]. In each tube 1.0 mL of hydatid cyst fluid was taken and mixed with 1.0 mL of each plant extract in comparison with the control group that is not treated with any plant extract. The rate of mortality was observed after specific time intervals 10, 20 and 30 min of exposure, and all experiments were performed in triplicates and data were derived from the mean of all the three replications. Percentage mortality of the dead protoscolices was measured by using the following formula, % mortality = $\text{ODP} \div \text{TP} \times 100$

2.4 Phytochemical Analysis

The phytochemical test of crude alcoholic extract of all plants was carried out by chemical tests. Methods described in the literature were used for qualitative [24] and quantitative screening [24, 25] of alcoholic extracts, to check the presence and quantity of bioactive phytoconstituents including Flavonoids, Alkaloids, Tannins and Saponins. All chemicals used for the phytochemical analysis were purchased from (Thermo Fisher Scientific, USA and Merck, Darmstadt, Germany).

2.5 Qualitative phytochemical analysis

2.5.1 Test for Flavonoids

2 mL of each extract, were added with few drops of 20% sodium hydroxide, yellow color appeared. After the addition of few drops of 70% hydrochloric acid, the yellow color disappeared. This appearance and disappearance of yellow color indicated the presence of flavonoids in the plant extract [24].

2.5.2 Test for Alkaloids

One mL of Marquis Reagent was added to 1 mL of sample along with few drops 40% formaldehyde and 2 mL of concentrated sulfuric acid and mixed; the dark orange or purple color appeared that shows the presence of alkaloids [24].

2.5.3 Test of Tannins

The appearance of brownish black or blue color shows the presence of tannins, by the addition of 10% ferric chloride to 2 mL of extract sample [24].

2.5.4 Test for Saponins

To 2 mL of plant extract, 6 mL of distilled water was added and shaken vigorously. The formation of bubble or persistent foam indicated the presence of saponins [24].

2.6 Quantitative Phytochemical Analysis

2.6.1 Total flavonoids content determination

Each plant sample (4g) were extracted repeatedly with 40 mL of 80% aqueous methanol, at 37°C, filtered, transferred to crucible, kept in water bath, evaporated to dryness and weighed [25].

2.6.2 Total alkaloids content determination

One gram of extract sample was mixed with 40 mL of 10% acetic acid in ethanol, covered and allowed to stand for 4 hours, filtered and filtrate was kept in water bath to concentrate ($1/4^{\text{th}}$ of its original volume). To this concentrated extract, ammonium hydroxide was added drop wise until the precipitation was complete. This solution was allowed to settle down and precipitate was washed with ammonium hydroxide, filtered, dried and weighed [24].

2.6.3 Total tannins content determination

The tannins content was measured by using Folin and Ciocalteu method with slight modification [24]. Distilled

water (3.75 mL) was added to each sample extract, along with 0.25 mL of Folin-Ciocalteu's phenol reagent and sodium carbonate solution (0.5 mL). The absorbance was measured using a spectrophotometer (UVmini-1240, Shimadzu, Japan) at 725nm and tannic acid dilutions were used as standard solutions.

2.6.4 Total saponins content determination

Four grams of each plant sample was suspended in 40 mL of 20% ethanol; the obtained suspension was kept in a water bath for 4 hours at about 50°C and stirred continuously. The obtained mixture was again re-extracted with another 40 mL of 20% ethanol and kept in a water bath at 90°C. Afterward, 4 mL of diethyl ether was added, shaken and the aqueous layer was recovered. 12 mL of n-butanol was added and washed twice with 2 mL of sodium chloride. The remaining solution was heated in water bath, after evaporation the samples were dried and weighed [25].

2.7 Antioxidant activity

For the evaluation of antioxidant potential of all the three alcoholic extracts at different concentrations (100, 200, 300 and 400 g/mL), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used. The plant extract samples were incubated with freshly prepared purple colored 3 mL DPPH (0.004%) solution that turned yellowish after 30 min at room temperature, measured by using a spectrophotometer (UVmini-1240, Shimadzu, Japan) at 514 nm. One mL of methanol alone served as blank and was replaced by samples in control test. The following equation was used for the calculation of % inhibition [26, 27].

$$\% \text{ inhibition} = \frac{\text{Abs of control} - \text{Abs of Sample}}{\text{Abs of control}} \times 100$$

2.8 Statistical analysis

The obtained data was analyzed by one-way analysis of variance (ANOVA). All analysis was accomplished by using Minitab software, version 16.

Ethical approval: The conducted research is not related to either human or animal use.

Table 2: Antiprotoscolices effect of *B.wallichiana* extracts at different concentrations and exposure times.

Concentra- tions	Experi- ments	10 min			30 min			50 min		
		Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)
10mg/ml	1	99	29	29.29	99	35	35.35	100	40	40.00
	2	97	28	28.86	96	34	35.41	100	39	39.00
	3	97	28	28.86	97	33	34.02	96	40	40.00
	Total	293	85	29.01	292	102	34.93	296	119	40.20
30mg/ml	1	97	35	36.08	97	42	43.29	98	52	53.06
	2	96	38	39.58	96	44	45.83	95	50	52.63
	3	98	38	38.77	98	47	47.95	96	54	56.25
	Total	291	111	38.14	291	133	45.70	289	156	53.97
50mg/ml	1	98	45	45.91	100	55	55.00	100	65	65.00
	2	100	47	47.00	99	59	59.59	97	62	63.91
	3	99	44	44.44	97	57	58.76	97	67	69.07
	Total	296	136	45.94	296	171	57.77	294	194	65.98
Control	1	96	11	11.45	99	16	16.16	96	21	21.87
	2	96	13	13.54	97	18	18.55	97	23	23.71
	3	99	13	13.54	97	17	17.52	100	23	23.00
	Total	288	37	12.84	293	51	17.40	293	67	22.86

3 Results

The fertility and reproducibility of the hydatid cysts is determined by the presence of free protoscolices in wet mount of hydatid cyst fluid. In the current study, the viability of protoscolices was assessed by using eosin stain (0.1% aqueous), prior to the experiment. Non-viable protoscolices get colored by staining and appear red/purple however viable protoscolices remain colorless and show amoeboid like movement and flame cell activity under light microscope. Untreated protoscolices remain viable for 1 hour (60 minutes) prior to experiment, with no significant difference between 0 and 60 min. After being incubated with in sterile preservative solution (negative control), the protoscolices remain viable for an average of 24 hours without treatment, with minimum differences in distinct movement, order of hooks and membrane integrity of protoscolices. However the microscopy of dead protoscolices showed distortion and degeneration in their morphology and structure. That further leads to the loss of motility, loss of hooks or presence of free hooks and calcareous capsules.

3.1 *In-vitro* treatment of protoscolices of fertile cysts

The mortality rate of protoscolices at different concentrations and various exposure times of ethanolic extracts of these plants have been shown in tables 2-4. The results displayed highest mortalities (97.92%) of protoscolices with alcoholic extract of *B. vulgaris* at concentrations of 50mg/ml after 30 min of exposure (Table-3). While results showed a significant effect when protoscolices of *E. granulosus* was treated with alcoholic extracts of *B. wallichiana* (45.94%, 57.77% and 65.98%) at given concentration of 50 mg/ml by following treatment periods of 10, 20 and 30 min, respectively (Table-2). Furthermore, *E. heliscopia* showed the mortalities 42.28%, 52.36% and 61.22% at concentration of 50 mg/ml after exposure duration of 10, 20 and 30 min respectively (Table-4). Different alcoholic extracts of plants show different efficacy against viability of protoscolices by subsequent exposure in different time duration.

Table 3: Antiprotoscolices effect of *B. vulgaris* extracts at different concentrations and exposure times.

Concentra- tions	Experi- ments	10 min			30 min			50 min		
		Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)
10mg/ml	1	100	32	32.00	99	45	45.45	100	56	56.00
	2	100	41	41.00	99	45	45.45	100	59	59.00
	3	98	37	37.7	96	47	48.95	98	60	61.22
	Total	298	110	36.91	294	137	46.59	298	175	58.72
30mg/ml	1	96	58	60.41	98	65	66.32	97	79	81.44
	2	98	62	63.26	99	69	69.69	97	77	79.38
	3	98	62	63.26	100	71	71.00	97	77	79.38
	Total	292	182	62.32	297	205	69.02	291	233	80.06
50mg/ml	1	100	72	72.00	100	85	85.00	95	93	97.89
	2	100	78	78.00	96	88	91.66	96	94	97.91
	3	95	77	81.05	96	88	91.66	98	96	97.95
	Total	295	227	76.94	292	261	89.38	289	283	97.92
Control	1	96	9	9.37	98	16	16.32	99	25	25.25
	2	98	11	11.22	97	19	19.58	98	22	22.44
	3	98	8	8.16	100	17	17.00	98	26	26.53
	Total	292	28	9.58	295	52	17.62	295	73	24.74

3.2 Phytochemical Analysis

3.2.1 Qualitative Phytochemical analysis

Qualitative analysis of alcoholic extracts of plants, *B. vulgaris* and *E. Heliscopia* shows presence of all the investigated constituents, while phytochemical analysis of *B. Wallichiana* indicates presence of flavonoids, alkaloids and saponins however tannin is absent (Table-5).

followed by *E. Heliscopia* (0.02±0.00%) and *B. wallichiana* (0.02±0.00%) respectively. Highest tannin and saponin quantity was recorded in plant *B. vulgaris* (2.12±0.02%) and *E. Heliscopia* (0.27±0.01%) respectively (Table 6). Phytochemical constituents are actually non-nutritive to plants and they have protective or disease preventive qualities that are considered beneficial to plants and human health.

3.2.2 Quantitative analysis of selected medicinal plants

Quantitative analysis of pharmacologically important phytochemicals revealed that all the medicinal plants contain these constituents in varying amounts in their leaves. The quantity of flavonoids was higher in *B. vulgaris* (4.69±0.13%), followed by *B. wallichiana* (0.65±0.01%) and *E. Heliscopia* (0.14±0.00%) respectively. Similarly component alkaloid was high in *B. vulgaris* (0.10±0.00%)

3.3 Antioxidant Activities

Antioxidant activities of ethanol extracts of *B. Wallichiana*, *B. vulgaris* and *E. heliscopia* are tabulated in Table 7. The antioxidant activities increase with increasing order of concentration of the samples as shown in Table-7. The highest antioxidant potential noted at 400 g/mL is 69.13 for *B. Wallichiana* followed by 59.80 *B. vulgaris*. All the three extract were found active.

Table 4: Antiprotoscolices effect of *E.heliscopia* extracts at different concentrations and exposure times.

Concentra- tions	Experi- ments	10 min			30 min			50 min		
		Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)	Total pro- toscolices	Dead pro- toscolices	Mortality rate (%)
10mg/ml	1	100	20	20	97	30	30.92	99	38	38.38
	2	100	23	23	99	32	32.32	97	35	36.08
	3	96	24	25	97	30	30.92	97	37	38.14
	Total	296	67	22.63	293	92	31.39	293	110	37.54
30mg/ml	1	98	30	30.61	97	41	42.26	97	48	49.48
	2	95	35	36.84	96	41	42.70	96	48	50.00
	3	96	34	35.41	98	45	45.91	98	51	52.04
	Total	289	99	34.25	291	127	43.64	291	147	50.51
50mg/ml	1	100	40	40.00	100	49	49.00	100	59	59.00
	2	98	43	43.87	99	53	53.53	96	60	62.00
	3	100	43	43.00	97	53	54.63	98	61	62.24
	Total	298	126	42.28	296	155	52.36	294	180	61.22
Control	1	99	10	10.10	95	18	18.94	96	20	20.83
	2	97	14	14.43	96	19	19.79	97	23	23.71
	3	100	15	15.00	100	17	17.00	97	22	22.68
	Total	296	39	13.17	291	54	18.55	290	65	22.41

Table 5: Qualitative phytochemical analysis of the plant species studied using alcoholic plant extracts.

Plants	Flavonoids	Alkaloids	Tannins	Saponins
<i>B. vulgaris</i>	+	+	+	+
<i>B. Wallichiana</i>	+	+	-	+
<i>E. Heliscopia</i>	+	+	+	+

Positive (+) shows presence, Negative (-) shows absence

Table 6: Quantitative estimation of medicinally important secondary metabolites in the plant taxa studied.

Plants	Flavonoids (%)	Alkaloids %	Tannins %	Saponins %
<i>B. vulgaris</i>	4.69±0.13	0.10±0.00	2.12±0.02	0.15±0.04
<i>B. Wallichiana</i>	0.65±0.01	0.02±0.00	0.00±0.00	0.27±0.02
<i>E. Heliscopia</i>	0.14±0.00	0.02±0.00	2.04±0.03	0.27±0.01

Table 7: Antioxidant activities of the plant species studied using alcoholic plant extracts.

Names	100 (µg/mL) %	200 (µg/mL) %	300 (µg/mL) %	400 (µg/mL) %
<i>B.Wallichiana</i>	35.65	45.12	53.19	69.13
<i>B. vulgaris</i>	41.21	52.11	57.86	59.80
<i>E. heliscopia</i>	34.21	35.43	41.21	48.32

4 Discussion

CE continues to be a major health problem in many countries and declared as emerging and re-emerging infection, despite of some progress in control of CE [27]. Surgery is still considered as the most effective treatment, however many drugs are being used for treatment but the problem is, metabolites of certain drugs are potentially toxic in some aspects. There is a need for natural treatment and therapies, instead of synthetic drugs that cause severe complications. To date many scolical

drugs have been reported but there has been no ideal drug having effectiveness as well as safeness [28]. Intense side effect of hypertonic saline, silver nitrate, cetrimide, ethyle alcohol and albendazoe sulfoxide at some concentrations are also documented [29]. The viability of protoscolices is mandatory for *in-vitro* and *in-vivo* study, which can be access by use of different vital as well as avital stains i.e. neutral red, Geimsa stain, Zeil Neilson, eosin, methylene blue and Trypane blue [30]. In current study 0.1% stain was used for staining o get access the viability of protoscolices. The non-viable protoscolices were appear red in color with eosin dye while viable were appear colorless. The antimicrobial and antiparasitic potential of many plants have already been well explored against wide range of harmful microbes and parasites, by using diverse procedures. The current study shows that *B.vulgaris* indicate highest activity against viability of protoscolices, followed by *B.wallichiana*, *E.heliscopia* at 10mg/ml, 30mg/ml and 50mg/ml after exposure of 10min, 20min and 30min. Similar considerable attention to alternative therapies, particularly using natural sources derived compounds for the treatment of hydatid disease, previously many studies related to the current study have been reported, including *in-vitro* scolicidal effect of aqueous extract of *Olea europaea*, S. [31], *Z. multiflora* [32], *S. ebulus* [33], *Z. officinale* [34], Musk [35].

Phytochemicals are non-nutritive plant constituents which have protective properties from diseases which are considered to be beneficial to human health. In the current study both qualitative and quantitative phytochemical analysis of selected medicinal plants has been carried out. Qualitative phytochemical analysis shows the presence of active compounds including alkaloids, flavonoids, tannins, phenol and saponins. *B. vulgaris* shows the highest quantity of alkaloids (0.107 ± 0.001), flavonoids (4.697 ± 0.135), tannins (2.123 ± 0.025) and saponins (0.156 ± 0.041), thus showing highest efficacy against viability of protoscolices. All these classes of compounds have good antioxidant potential. Flavonoids are able to inhibit the initiation, promotion and progression of tumors, reduce coronary heart disease due to antioxidant effects [36, 37]. The mechanism of action of flavonoids is through chelation and its effect on membrane permeability as well as act on membrane bound enzymes [38]. The phenolic compounds have also important scavenging ability due to hydroxyl group [39]. Similarly, saponins are less toxic to mammals and have antimicrobial activity, highly used as mild detergent [40]. Moreover, alkaloids have a broad range of medicinal activities including anticancer, antiasthma and antimalarial [41], vasodilatory [42], analgesic [43] and antibacterial properties [44].

5 Conclusion

It is concluded that all the selected plants including *B. Wallichiana*, *B. vulgaris* and *E. heliscopia* shows antiprotoscolices effect against protoscolices of *E. granulosus*. Therefore it is recommended that these plants could be used as complementary treatment after investigation on the animal models and clinical trials in human population.

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