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An Evaluation of the Antibacterial and Antiviral Activities of Some Bryophytes

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THIS STUDY screen the antibacterial and antiviral activities of some bryophytes extracts. The pathogenic bacteria *Listeria monocytogenes* LMG 10470 (*L. monocytogenes*), *Escherichia coli* LMG 8223 (*E. coli*), *Bacillus cereus* ATCC 14579 (*B. cereus*) and *Pseudomonas aeruginosa* LMG 8029 (*P. aeruginosa*) were inhibited by the aqueous methanolic extracts (ME) of *Imbibryum* sp., *Barbula convoluta* and *Trichostomum* sp.. The mixture of *Imbibryum* sp.extract and tetracycline have synergistic effect against *P. aeruginosa* while the mixing of *Trichostomum* sp. extract with tetracycline has antagonistic effect against *P. aeruginosa.*Scanning Electron Microscopy (SEM) of *P. aeruginosa*, treated with ME of *Barbula convolute*, *Imbibryum* sp. and *Trichostomum* sp.indicated sheath surrounded the bacteria, signs of irregular wrinkled outer surface, adhesion and aggregation of damaged cells, malformations in bacterial shape compared to untreated bacterial control. The bryophyte species screened exhibiting considerable antiviral activity against zucchini yellow mosaic virus (ZYMV), so the bryophytes have been identified as a new source of antiviral activity. The highest degree of antiviral activity was shown ME of *Barbula convoluta*, *Imbibryum* sp. and *Trichostomum* sp.against ZYMV (94, 92 and 90%, respectively). The tested cucumber plants which mechanically infected by ZYMV and treated with different extracts of the most potent bryophytes (*Imbibryum* sp), contain high amount of phenolic compounds. The highest contents of total phenol are detected in infected cucumber plant, which treated with benzene extract of *Imbibryum* sp.,(3.48 mg/g fresh wt) followed by methanol extract (3.19 mg/g fresh wt). The bryophytes extracts have no toxic effect in Wistar Albino rats.

Keywords: Bryophytes, Antibacterial, Antiviral, Cucumber, Pathogenic bacteria, ZYMV, Physiological analysis.

Introduction

Bryophytes (mosses) represent the second largest group of green land plants after angiosperms, are taxonomically placed between algae and pteridophytes (Asakawa, 2007 and Tedela et al., 2014). Bryophytes posses medicinally important bioactive compounds but with little information. Bryophytes used throughout the world as drugs and remedies to cure the various diseases (Bodade et al., 2008 and Sabovljević et al., 2016).

There are more than 22.000 members of the mosses are existing in the world (Zinsmeister & Mues, 1987). Although Bryophytes are very familiar, their medicinal importance is not exploited completely. They are used in pharmaceutical products, horticulture and household purposes (Kumar et al., 1999). Bryophytes treat illness of cardiovascular system, tonsillitis, bronchitis, skin diseases and burns. They also possess anticancer and antimicrobial activity due to their unique chemical constituents (Banerjee & Sen, 1979 and Askawa, 1990). *Plagiochila fasciculate* (member of Bryophytes) shows inhibitory effect on virus (*Herpes simplex type 1, Polio type 1*) and bacteria (*Bacillus subtilis, E.coli, Candida albicans and Cladosporium resinae*) (Lorimeres & Perry, 1994). Both acetone and ethanol extracts of the bryophytes inhibited the growth of *Escherichia coli, Bacillus cereus, Erwinia chrysanthemi* and *Pseudomonas aeruginosa* on an agar plate

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(Kandpal et al., 2016). In fact, Bryophytes have been proven to be apotent, nontoxic and broad spectrum antibacterial substances (Lashin et al., 2015).

Bryophytes are considered as a "remarkable reservoir" of new, natural products or secondary compounds, many of which have shown interesting biological activity. To date, over several hundred new compounds have been isolated from bryophytes and their structures have been elucidated. Among the flavonoids examined, four flavonols (myricetin, datiscetin, kaempferol and quercetin) and two flavones (flavones and luteolin) exhibited inhibitory activity against methicillin-resistant *Staphylococcus aureus* (MRSA). Seven pure flavonoids were isolated and identified from five moss species (Basile et al., 1999). All the flavonoids showed good antimicrobial activity against the tested bacteria and the highest activity that of saponarine. Some of these flavonoids were shown to have pronounced antibacterial effects. Biflavonoids in mosses have also been reported as possible agents against microorganisms (Lopez-Saez, 1996).

Viruses are unlike any other pathogen. In fact, viruses are very complex chemical molecules which depend entirely on host cell machinery to reproduce (Webster's et al., 1998). Zucchini Yellow Mosaic Virus (ZYMV) is a Potyvirus with a worldwide distribution (Murphy et al., 1995). The flexuous filamentous particles, 750 nm long (Lisa et al., 1981), consists of single-stranded RNA about 9600 nucleotide long (Balint et al., 1990). Blua & Perring (1989) showed that early ZYMV infection can cause 94% reduction of marketable cantaloupe. Antiviral activity occur in a wider variety of plants including ferns and liverworts, and the more properly byrophyta, pteridophyta and spermatophyta (Desselberger, 1995). There are no reported cases of viruses capable of infecting bryophytes and thus it seems quite possible that bryophytes contain a chemical defense against viruses. A large majority of the bryophyte species have been identified as exhibiting considerable antiviral activity against PVX, the bryophytes have been identified as a new source of antiviral, are a rich source of secondary metabolites with antimicrobial activities. Recent investigations of antiviral compounds have suggested that bioflavonoid reported in bryophytes cause a powerful inhibition to a broad spectrum of viral pathogen (Hillhouse, 2003). The secondary metabolites identified from mosses belong to terpenoids, flavonoids and bibenzyls (Asakawa, 1981 and Kothyari, 1997). Terpenoids, phenolic and volatile constituents have also been investigated in some bryophytes. Many of the terpenoids, flavonoids and alkaloids were described and isolated mainly from liverworts (Saritas et al., 2001 and Chaudhary & Kumar, 2011). The antibacterial activity of flavonoids has been reported (Singh & Bhat, 2003).

Asakawa (1990, 2001) and Asakawa et al. (2000) stated that almost all species of bryophytes are not damaged by insect larvae, fungi, bacteria, slugs, snails and mammals because, biological compounds like oligosaccharides, polysaccharides, sugar alcohols, amino acids, fatty acids, aliphatic compounds, phenyl quinone and aromatic phenolic substances in bryophytes are protected against these organisms. It is well known that plant phenols, particularly the free phenols (whichare toxic substances) play a significant role in controlling pathogenic microorganisms attacking some variety of plants. Unlike situation in the non inducted plants, the plants inducted by either biotic or abiotic inducers contained higher levels of sugars and phenols (Meena et al., 2001).

An evaluation of toxic properties of a substance is crucial when considering for public health protection because exposure to chemicals can be hazardous and results to adverse effects on human being. In practice, the evaluation typically includes acute, sub-chronic, chronic, carcinogenic and reproductive effects (Asante-Duah, 2002). The long term safety level of a compound can be predicted from acute or shorter than sub acute studies (Ministry of Health and Welfare, 1977) and (Perry, 1971). The purpose of toxicity testing is to provide adequate database to make decisions concerning the toxicology properties of chemical and commercial products. In some situations, the purpose is to decide whether a material will be safe. Under the conditions of expected use in other situations, the objective is to establish the safe limits in condition of use. Lashin et al., (2015) showed that the aqueous methanolic extracts of *Imbibryum* sp., is not toxic in male and female Wistar rats, suggesting a safety use by humans.

The present investigation was aimed to study the antibacterial, antiviral, phytochemical and potential toxicity of secondary metabolites of some bryophytes (mosses).

Materials and Methods

Collection of bryophytes and solvent extracts preparation: Eight mosses plants were collected from different habitats during 2013. The specimens were identified according to Lashin (2011), Wijk et al. (1992), Smith. (1994) and Terry (2007) in Botany Department, Faculty of Science, Zagazig University, Egypt. Solvent extracts preparation were done according to Nikolajeva et al. (2012) with some modification. At first, plants were washed with steriledistilled water then dried, powdered and extracted (10 g/100mL) with different solvents: acetone, ethanol, methanol, benzene and petroleum ether and dried in vacuum till dried (48 h). The aqueous extract obtained were filtered, centrifuged at 3000 rpm for 10 min. then, sterilized aqueous extracts were used.

Tested microorganisms

Four bacterial species were *Listeria monocytogen* LMG 10470 (*L. monocytogenes*), *Pseudomonas aeruginosa* LMG 8029 (*P. aeruginosa*), *Bacillus cereus* ATCC 14579 (*B. cereus)* and *Escherichia coli* LMG 8223 (*E. coli)* were from the Microbiology Lab in Faculty of Science Zagazig University. All above bacteria were maintained onto brain heart infusion (BHI) agar medium (Oxoid Ltd, UK).

Screening of antibacterial activity of mosses extracts

Sterilized discs of filter paper (6 mm diameter) were soaked in 1 mL of each extracts of bryophytes, separately, for 2 min and then used for screening. Nutrient agar was used as basal medium. The inoculated plates were incubated at 37°C for 24 h. After incubation, inhibition zone diameters of discs for each treatment were measured to the nearest millimeter (mm) (Saeed et al; 2007). The bryophytes (*Barbula convoluta, Imbibryum* sp. and *Trichostomum* sp.) showed the highest inhibition against tested bacteria so they were chosen for further study.

Antibacterial activities of antibiotics, Bryophytesextracts-antibiotics combination and Bryophytes only by disc diffusion assay

Hundred µL of bryophytesextract (*Barbula convoluta, Imbibryum* sp. *and Trichostomum* sp.) and the antibiotic were loaded on sterilized discs with different concentration (90% bryophyteextract+ 10% antibiotic, 50% ml bryophyteextract+ 50% ml antibiotic and 10% ml bryophyteextract + 90% ml antibiotic) in petri-dishes with brain heart infusion (BHI) agar medium were prepared. The sterilized filter paper discs were soaked in the above mixture till saturation, then incubate for 24 h. at 37º C.

Scanning electron microscopy (SEM) analysis

SEM was performed according to Benli et al. (2008) with some modification, to further explain the mode of action of the studied mosses on bacterial cell morphology. An aliquot of 0.1 mL of *P.aeruginosa* culture (the most sensitive) was inoculated into 10 mL nutrient broth and incubated at 37 °C with gentle agitation for 12 h. The cells were collected at 4500xg for 15 min at 4 °C. Cells were washed with PBS three times and resuspended in PBS (pH 7.4) at the same volume. The antimicrobial agents (100 µL) were added to the cell suspension and incubated at 37 °C with gentle agitation for 4 h. The control sample was prepared similarly but without treatments. Bacterial cells were recovered by centrifugation at 4500xg for 15 min at 4 °C, washed with PBS (pH 7.4) and fixed in 2.5% glutaraldehyde in PBS. The fixed bacterial pellet was then dehydrated in graded alcohol series, dried and mounted onto stubs using double sided carbon tape, coated with thin layer of gold. All cell samples were examined in Scanning Electron Microscope (JEOL-SEM, JAPAN).

Antiviral activities of bryophytes extracts:

Zucchini Yellow Mosaic Virus (ZYMV) was prepared from samples previously identified by (Abdel-Shafi, 2005). The cotyledonary leaves and first leaf of host cucumber plants (*Cucumis sativus* L.) were dusted with carborundum (600 mesh, prolab), then mechanically inoculated with the virus inoculum by clean finger. The inoculated leaves were washed with distilled water according to Yarwood (1955). The infected leaves of cucumber (developing mosaic, blistering, malformation after 21 days of inoculation) were frozen in a deep freeze until used.

In vitro studies (aqueous solvents extracts of bryophytes mixed with viral sap)

In this experiment, equal volumes of aqueous solvent extracts of bryophytes and viral sap were mixed together for 30 min. (2 ml of sap containing virus + 2 ml of bryophytes extracts in test tubes) and then inoculated directly. Cotyledonary leaves and first leaf of *Cucumis sativus* plant were inoculated with 100 µL of the mixture after dusting the leaves with carborundum

(600 meshes, prolab), then the inoculated leaves were washed with distilled water according to Yarwood (1955). The symptomatic plants showed symptoms like mosaic, green blisters and maleformation were counted after 21 days and the mean of 20 plants per each treatment was calculated. General control plants (healthy plants) were inoculated with buffer only. Viral control plants were inoculated by ZYMV only. The percentage of inhibition calculated according to the equation :

% of viral inhibition = number of symptomatic plants in viral control – number of symptomatic plants in treatment / number of symptomatic plants in viral control X 100.

Plants were harvested and then number of leaves, shoot length and fresh weight were determined.

In vivo studies

Pre- inoculation experiment (treatment with bryophytes extracts before virus infection):

ZYMV inoculum (100 µL) was inoculated on the treated leaves with bryophytes extracts after 24, 48 and 72 h. The inoculated leaves were then washed with distilled water. Controls include virus infected plants (viral control) and general healthy plants (general control) were done. The number of symptomatic plants were counted after 3 weeks for each time intervals and % of inhibition were calculated. Plants were harvested and then number of leaves, shoot length and fresh weight were determined.

Post-inoculation experiment (treatment with bryophytes extracts after virus infection)

The cotyledonary leaves and first leaf of *Cucumis sativus* L. plants were inoculated with virus inoculum (100 µL / leaf) after dusting the leaves with carborundum, then the inoculated leaves were washed with distilled water and treated with bryophytes extracts after 24, 48 and 72 h of inoculation. The developing symptoms were recorded after 21 days (20 plants for each treatment) and % of inhibition were calculated. The viral control and healthy control were done. Plants were harvested and then number of leaves, shoot length and fresh weight were determined.

Physiological analysis

Estimation of photosynthetic pigments

The mosses photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined by using spectrophotometric method described by Metzener et al. (1965) a known fresh weight (1.0 g) of mosses samples was homogenized in cold 85% acetone for 5 min in a dim light. The homogenate was centrifuged and the supernatant extract was made up to an appropriate volume with 85% aqueous acetone. The colour intensity was measured against a blank of 85% aqueous acetone at three different wave lengths of 452.5, 644 and 663 nm using spectrophotometer (Parkin-Elemer Lambda1-UV/VIS, U.S.A.) taking into consideration the dilution factor. The amount of each pigment fraction (chlorophyll a, chlorophyll b and carotenoids) was determined as mg/g fresh weight, using the following equations :-

Chlorophyll a = (10.3 E 663 - 0.918 E 644) x V/1000 x W

Chlorophyll b = (19.7 E 644 - 3.87E 663) x V/1000 x W

Carotenoids = 4.2 E 452.5 x V/ 1000 x W–(0.0264 Chl. a + 0.426 Chl.b)

where : E = the optical density.

V = the final volume of 85% acetone chlorophyll extract.

W = the fresh weight of green leaves of squash plants.

Estimation of phenolic compounds (Lallyatt, 1977)

Extraction of phenolic compounds

One gram of fresh weight of mosses samples were homogenized in 20 mL ethanol (80%) for 10 min. The homogenate was filtered and residue washed with 10 mL ethanol (80%). The filterate was shaken for 10 min with 40 mL petroleum ether (40-60 C) and partitioned by allowing standing for 10 min. The alcoholic phase was removed and shaken 3 times more with petroleum ether. Then were collected and dried down and dissolved in 5 mL distilled water.

Determination of phenolic compounds

Free bound and total phenols were determined spectrophotometrically at 520 nm, using Folin method as described by Snell & Snell (1953). Phenolic compounds were determined as mg/g fresh weight based on a standard curve for pyrogallol. The difference between the total phenols and free phenols is the value of bound (conjugated) phenols.

Estimation of total nitrogen and crude protein contents

The total nitrogen and crude protein contents were estimated by the micro-Kjeldahl method according to Allen (1953).

Reagents

1) Concentrated sulfuric acid.
2) Digestive mixture (Cole & Parkers, 1946). It consists of potassium sulphate: copper sulphate: selenium dioxide at a ratio by weight (10:1:0.5, respectively).
3) Mixed indicator: 8 mL of bromocresol green (0.1 w/v) in 95% ethyl alcohol and 1 mL of methyl red (0.1 w/v).
4) Sodium hydroxide 50 % (w/v).
5) Boric acid 4 % (w/v).

Procedures

Fresh weight of different plant samples (0.5 g) was transferred to 50 ml Kjeldahl flask and mixed with 2 mL sulfuric acid and 0.5 g of digestive mixture. The samples were digested until the formation of clear liquid free from black residues. The digested samples were left to cool and each sample was completed to 20 ml by distilled water, and then transferred to the distillation apparatus. 10 ml of sodium hydroxide (50 % w/v) were added via the stopped funnel. Produced ammonia was captured in 10 mL of 4 % (w/v) boric acid and mixed indicator till final volume of 50 mL. Titration was carried out by 1/70 N hydrochloric acid and the total nitrogen was calculated as mg/g dry weight.

The crude protein content was estimated by multiplying the total nitrogen content by a constant factor of 6.25 (Hojjati, 1976):

$$1 \text{ mL HCl } (N/70) \equiv 0.2 \text{ mg}$$

Absence of toxicity of bryophytes extract (Barbula convoluta, Imbibryum sp. and Trichostomum sp.) in Wistar albino rats

Animals

Healthy male and female white albino rats (*Rattus norvegicus*. Bork), Wistar strain (135 ± 10 g, body weight for female and 155 ± 15 g, body weight for male) were obtained from Organization of Biological Products & Vaccine (Helwan Farm, Cairo, Egypt) and housed in plastic cages in groups of 5 animals / cage. The experimental animals were allowed to acclimatize under the laboratory conditions (temperature of 25 ± 5 ˚C; relative humidity 50 – 70 % and normal light/dark cycle) for 2 weeks prior the experiment. They were provided with balanced pelleted diet (23 % protein) and tap water *ad libitum* throughout the adaptation and experimental period.

Experimental design

Experiment design included two phases:

-The first phase was to determine the acute oral medium lethal doses (LD50).

-The second phase was to assess the sub-acute toxicity of tested bryophytes.

Hematological analysis

Hematological analysis including white blood cell (WBC), red blood cell (RBC), platelet counts (PLT), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and lymphocyte (LYM) estimation were carried out using the SYSMEX hematology auto analyzer (Japan).

Histo-pathological examination

The organs of randomly selected three rats from each group were subjected to histo-pathological examination. Vital organs such as kidney and liver were excised, examined grossly and subsequently fixed in 15 % formalin saline. The fixed tissues were processed by dehydration in a series of graded ethanol concentrations, cleared with xylol and embedded in paraffin blocks. Sections of 4 µ thickness were obtained and stained by Hematoxilen - Eosin stain (H & E) for the histo-pathological analysis under light microscope (model OLYMPUS CX 41) at 1200 x magnification (Humason, 1979).

Statistical analysis

Data of the all trial alone were statistically analyzed using the General Linear Model Program of SAS (1996). Differences among means were tested by Duncan's multiple range test (Duncan, 1955).

Results and Discussion:

Control of bacterial and viral diseases is difficult through preventive or curative measures. More efforts have been directed towards a more durable and economical solution by new strategies. It was found that the biological control is the best mean that replace the chemical control (Kozo et

al., 1998). Moreover, it is important to find safe and cheap effective antimicrobial agents to inhibit virus and pathogenic bacteria. The bryophytes produce a great number of secondary metabolites, including terpenoids and polyphenolic compounds or nitrogen containing compounds many of which show interesting biological activity such as cytotoxicity, antiviral activity and antibacterial activity. (Asakawa et al., 2013 and Kandpal et al., 2016).

Identification of Bryophytes:

The current work was designed to isolate and identify eightmosses taxa which described in details according to gametophyte and sporophyte. All identified taxa have only gametophyte stage except *Funaria hygrometrica* has gametophyte carrying sporophyte according to Lashin (1990), Wijk et al. (1992), Smith (1994), Terry (2007) and Lashin (2011). The studied taxa were photographed and illustrated in Fig.1 (Plate 1-8) and Fig. 2.

Classification of studied mosses according to (Flowers, 1973)

Division: Bryophyta
Class: Bryopsida
Order: Bryales

Family 1: Fissidentaceae
- *Fissidens* sp. **Fig. 1 (Plate 1).**

Family 2: Pottiaceae
- *Trichostomum* sp.**Fig.1 (Plate 2).**
- *Didymodon* sp. **Fig. 1 (Plate 3).**
- *Barbula* sp. **Fig. 1 (Plate 4).**
Barbula convoluta **Fig.1 (Plate 5).**

Family 3: Funariaceae
Funaria hygrometrica **Fig.1 (Plate 6).**

Family 4: Bryaceae
- *Imbibryum* sp. **Fig. 1 (Plate 7).**
Splachnobryum obtusum **Fig.1 (Plate 8).**

Plant description

Fissidens sp.

Plants green to reddish brown. Leaf lamina cells iso diametric, quadrate, hexagonal to rounded, smooth, bulging, uni papillose to pluri papillose with simple, bifid, or c- shaped papillae; basal cells rectangular, smooth, bulging or uni papillose, some times hyaline and basal marginal cells extends up ward (Plate 1).

Trichostomum sp.

Plants female, green to dark green, large up to 1.6 cm high, stem branched or unbranched. Leaves lanceolate, apex acute, costa short excurrent, upper lamina cells quadrate, lower lamina cells rectangular, cells strongly papillose in mid leaf cross section (Plate 2).

Didymodon sp.

Plants female, green to olive green above, yellowish brown to reddish brown below, medium up to 0.6 cm, large up to 1 cm high, stem branched, semi rounded or angular in cross section. Leaves elongated triangular, apex a cute to obtuse, costa ending below apex by 2-5 cells (Plate 3).

Barbula sp.

Plants sterile, yellowish green to olive green, large up to 1 cm high. Stem usually un branched. Leaves apex acute, margins slightly re curved, costa per current, stem semi circular in cross section (Plate 4).

Barbula convolute

Plants female, yellowish green, large up to 1.3 cm high, stem branched or un branched. Leaves broadly lanceolate, apex broadly acute or obtuse, costa ending below apex by (1- 4) cells, upper lamina cells elongated (Plate 5).

Funaria hygrometrica

Plants female, yellowish green, medium up to 0.7 cm high, stem branched or un branched. Leaves ovate to lanceolate, costa ending below apex by (2-3) cells, margin toothed, upper lamina cells circular, lower lamina cells elongated (Plate 6).

Imbibryum sp.

Plants female, green to yellowish green, medium up to 0.7 cm high, stem branched or un branched. Leaves ovate to elongate, costa short ex current, lamina cells rhomboidal (Plate 7).

Splachnobryumobtusum (Brid.)

Plants *female*, yellowish green to dark green, medium up to 0.6 cm high, stem un branched, leaves ovate, apex obtuse, margins re curved, costa ending by (1-4) cells below apex, cells rhomboidal (Plate 8).

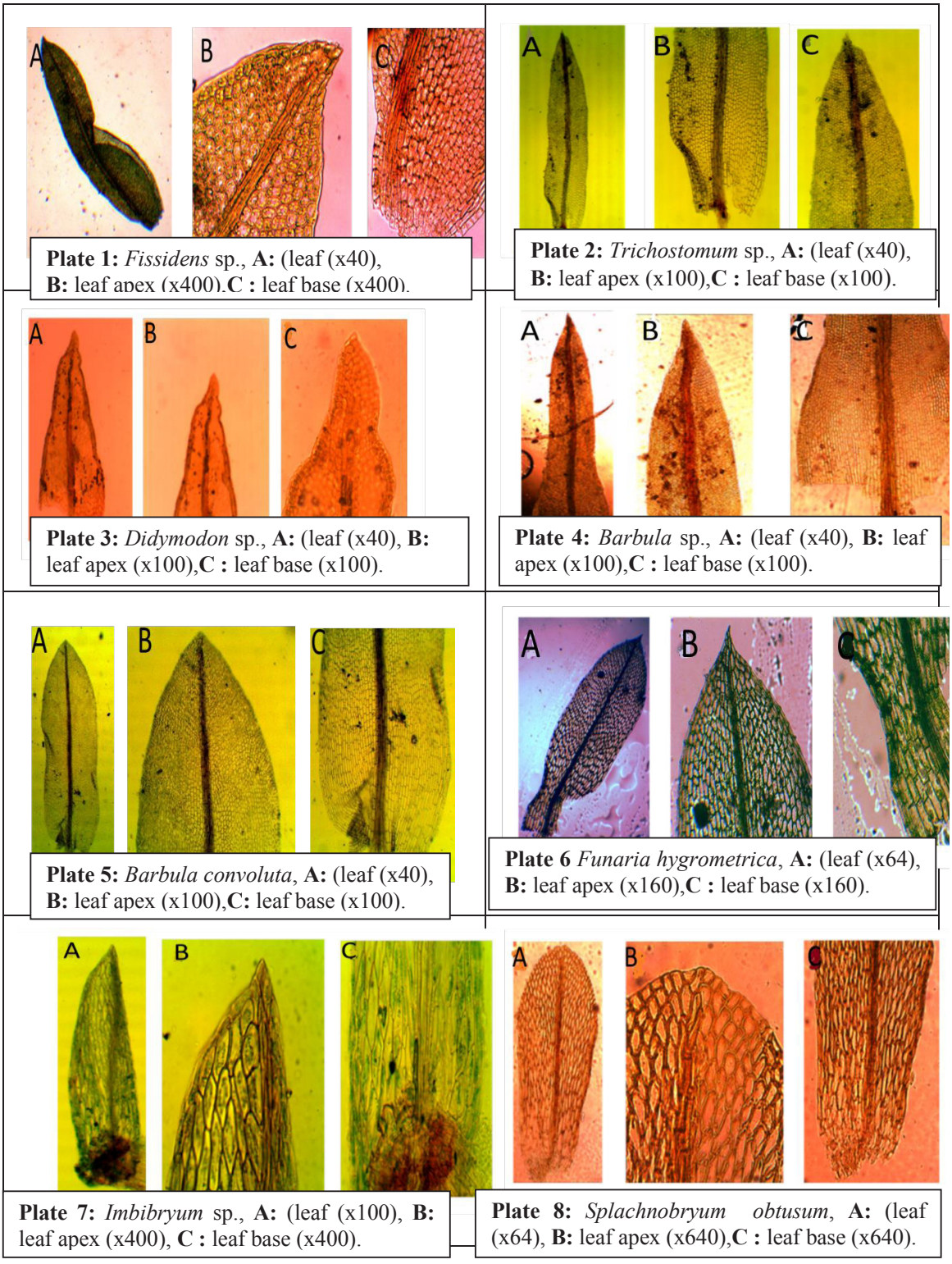


Fig. 1(Plate 1-8). Photographs of eight identified bryophytes (mosses) taxa as seen under light microscope.s.

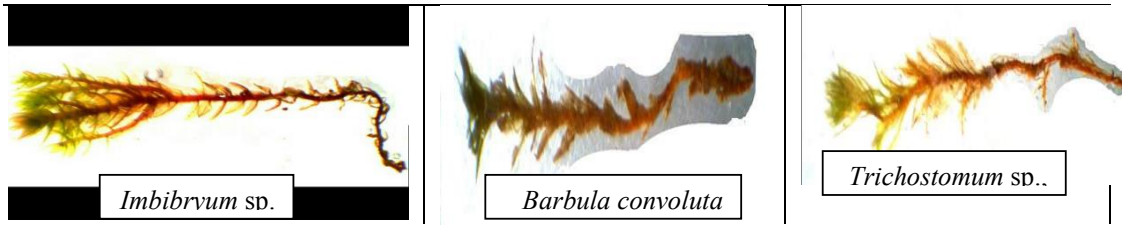


Fig.2. Photo of natural most potent antibacterial and antiviral bryophytes.

An evaluation of the antibacterial activities of the mixture of bryophytesextract and antibiotics

The methanolic extracts of *Imbibryum* sp. *Trichostomum* sp. *and Barbula convolute* were highly inhebited the pathogenic bacteria (*E. coli, B. cereus, L. monocytogenes* and *P. aeruginosa*), (Table 1). The mixture of *Imbibryum* sp. with tetracycline has inhibitory effect on *P. aeruginosa* as sensitive indicator bacteria (Table 2 and Fig. 3). The results showed that synergistic effect where with increasing the *Imbibryum sp.* concentration the inhibition zone diameter increase. However, the mixture of *Trichostomum sp*. with tetracycline has antagonistic effect on *P. aeruginosa* as sensitive indicator bacteria (Table 3 and Fig. 4). The results showed that with increasing the *Trichostomum* sp. concentration the inhibition zone diameter decrease. In this study The highest degree of antibacterial activity shown by the aqueous methanolic extracts of *Imbibryum* sp., *Barbula* convoluta and *Trichostomum* sp., against *P. aeruginosa* (the most sensitive bacteria for tested mosses).Generally bryophytes are known to possess extremely high amounts of terpenoids, phenolic (flavonoids and bi benzyl derivatives), glycosides, fatty acids and also some rare aromatic compounds. This result agree with that obtained by Elibol et al. (2011) who indicated that ethanolic and methanolic extracts of some mosses had inhibition effect against *E. coli.* and *Salmonella* while acetone extract was inactive against the tested bacteria. The results obtained showed that *Imbibryum* sp. extracts inhibited all tested bacteria and the most sensitive one was *P. aeruginosa* (40 mm inhibition zone). The present study revealed that the antibacterial activity of mosses extracts against Gram positive bacteria as well as Gram negative bacteria. This makes the advantages of selected Bryophytes as a wide range natural antimicrobial agent.This results agree with that obtained by Bodade et al. (2008). The antibacterial activity of mosses (*Imbibryum* sp., *Barbula convoluta* and *Trichostomum* sp.) could be due to some bioactive materials in these plants such as terpenoids, flavonoids and Saponines. This agree with Elibol et al. (2011).

Antimicrobial activity is related to the specific chemical composition, structural configuration of compounds, functional groups, as well as potential synergistic or antagonistic interactions between compounds. To date, over several hundred new compounds have been isolated from bryophytes and their structures have been elucidated. The biological characteristics of the terpenoids and aromatic compounds isolated from liverworts show antibacterial and antifungal activity, cytotoxic activity, anti HIV, insect anti feedant activity, and superoxide anion radical release activity (Asakawa, 2008 and Tedela et al., 2014). Seven pure flavonoids were isolated and identified from five moss species (Basile et al., 1999). All the flavonoids showed good antimicrobial activity against the tested bacteria Biflavonoids in mosses have also been reported as possible agents against microorganisms (Lopez-Saez, 1996).

Scanning electron microscopy (SEM) examination

SEM examination was used to show the changes of an overnight culture of *P. aeruginosa,* induced by the treatment with *Imbibryum* sp., *Trichostomum* sp. and *Barbula convoluta* for 4 h at room temperature (Fig. 5). Control bacterial cells (without any treatment) were morphologically regular, intact and typical. However, treated bacterial cells showed presence of outer sheath, signs of irregularity, wrinkled outer surface, fragmentation, and adhesion of damage cells and presence of outer sheath. There were different effects of each tested bryophytes against bacteria. *Barbula convoluta* showed strongly inhibition and male formation against (*E.coli, P. aeruginosa, B. cereus* and *L. monocytogenes*). These malformation in bacterial morphology were similar to that obtained by penicillin antibiotic (Sitohy et al., 2012). The previous reports referred to the rapid action of natural antimicrobial agents (Yount & Yeaman, 2005). Until our knowledge this is the first time of studying the effect of mosses on pathogenic bacteria under electron microscope.

TABLE 1. Antibacterial activities of aqueous methanol extracts of some mosses against Gram positive (*L. monocytogenes* and *B. cereus*) and Gram negative bacteria (*P. aeruginosa* and *E. coli*) using disc assay method .

Bacteria	Inhibition zone diameter (mm)		
	Imbibryum sp.	Trichostomum sp.	Barbula convoluta
L. monocytogenes	35	-	32
B. cereus	32	-	24
P. aerugenosa	40	33	30
E. coli	30	-	25

Values of inhibition zones are means of three replicates. (-): No inhibition.

TABLE 2. Antibacterial activity of mixing of *Imbibryum* sp. with antibiotic tetracycline against *P. aeruginosa* by disc diffusion method.

Concentration (µg ml^{-1})	Inhibition zone diameter (mm)
A= (Tetracycline) 100	40 ± 0.65
B= (20 Imbibryum sp. + 80 Tetracycline)	40 ± 0.44
C= (50 Imbibryum sp.+ 50 Tetracycline)	42 ± 0.59
D= (80 Imbibryum sp.+ 20 Tetracycline)	44 ± 0.82
E= (Imbibryum sp.) 100	36 ± 0.66

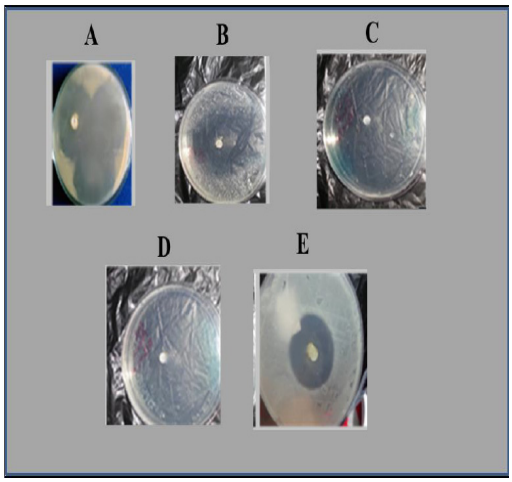


Fig. 3. Antibacterial activity of mixing of *Imbibryum sp.* and *Tetracycline* against *P. aeruginosa* (synergistic effect)

TABLE 3. Antibacterial activity of mixing of *Trichostomum* sp. with antibiotic tetracycline against *P. aeruginosa* by disc diffusion method.

Concentration (µg ml^{-1})	Inhibition zone diameter (mm)
A= (Tetracycline) 100	37 ± 0.65
B= (20 Trichostomum sp. + 80 Tetracycline)	37 ± 0.44
C= (50 Trichostomum sp. + 50 Tetracycline)	34 ± 0.59
D= (80 Trichostomum sp. + 20 Tetracycline)	32 ± 0.82
E= (Trichostomum sp.) 100	35 ± 0.85

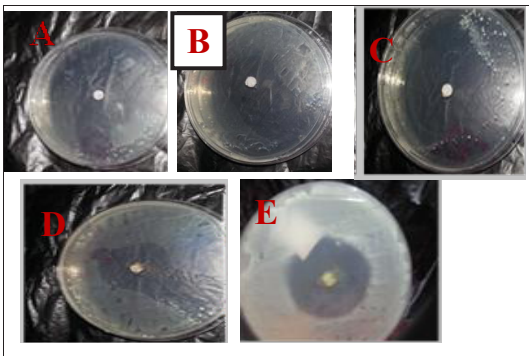
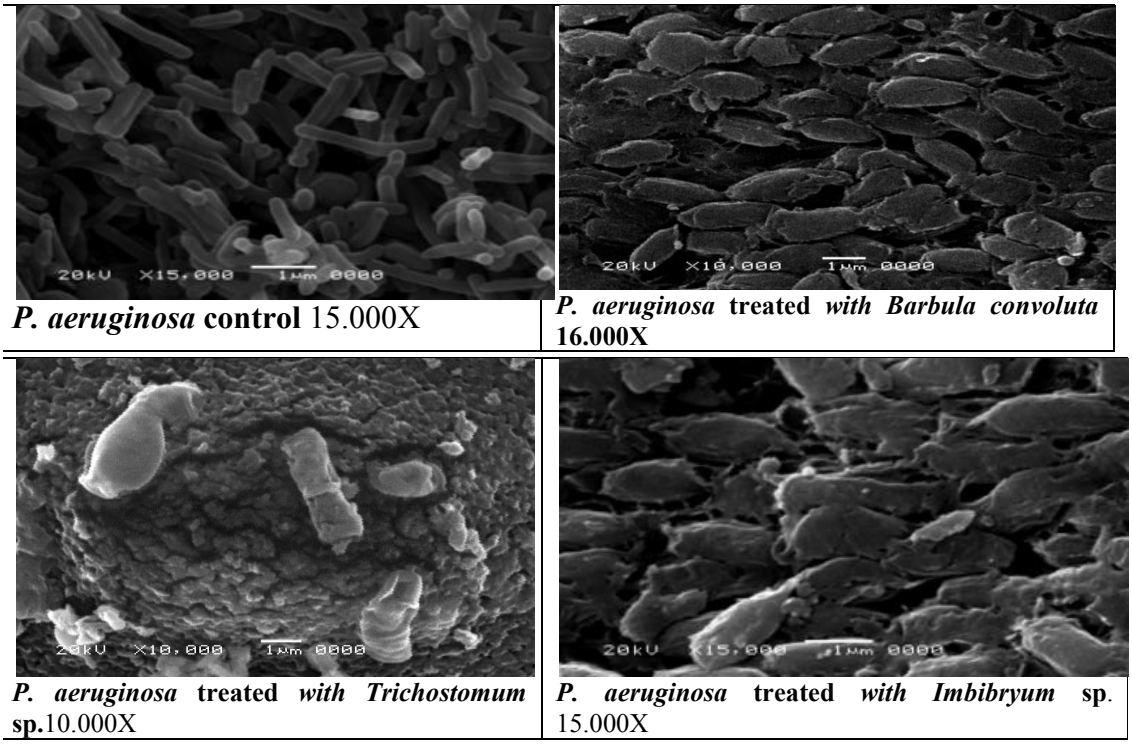


Fig. 4. Antibacterial activity of mixing of *Trichostomum* sp. and tetracycline against *P. aeruginosa*. (Antagonistic effect)



***P. aeruginosa* control** 15.000X

***P. aeruginosa* treated *with Barbula convoluta* 16.000X**

***P. aeruginosa* treated *with Trichostomum* sp.**10.000X

***P. aeruginosa* treated *with Imbibryum* sp.** 15.000X

Fig. 5. SEM of *P. aeruginosa* treated with *Barbula convolute, Imbibryum* sp. and *Trichostomum* sp. compared to untreated control bacteria.

Antiviral activities of bryophytes extracts

The virus was then propagated and maintained in *Cucumis sativus* plants. The virus showed symptoms like green blisters, leaf roll and mosaic (Fig.6)

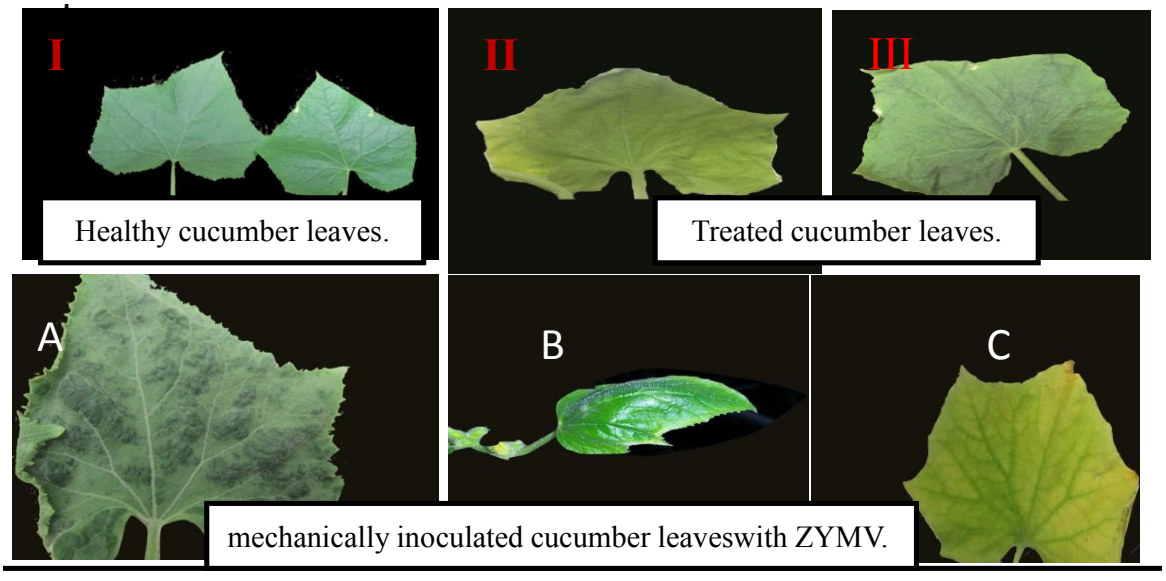


Fig. 6. I) Healthy leaves; II) Treated with Barbula convulata ; III) treated with *Imbybrium* sp. Cucumber leaves mechanically inoculated with Zucchini Yellow Mosaic Virus (ZYMV). The leaves showed ZYMV symptoms. A: green blisters, B: leaf roll and C: mosaic and yellowing.

In vitro screening of the antiphytoviral activities of bryophytes extracts

The aqueous ethanol, methanol, benzene, petroleum ether and acetone extracts of eight mosses were screened for their antiviral activities against ZYMV (Table 4). The methanolic extracts of , *Barbula convoluta, Imbibryum* sp., and *Trichostomum* sp. showed the highest inhibition of ZYMV (94, 92 and 90 respectively) (Table 4).Therefore, they were chosen for further study. The morphological criteria of treated cucumber plants with bryophytes extractswere represented in Table 5), increase the cucumber growth.The obtained data revealed that foliar application of bryophytes extracts increase the growth of the plants more than the viral control and inhibit the viral symptoms (Fig. 6). All extracts showed increase in shoot length and fresh weight at all time intervals compared to viral control.

TABLE 4. Effect of bryophytes extracts on the infectivity of ZYMV on cucumber plants (*in vitro*) (2 ml of ZYMV sap + 2 ml of aqueous bryophytes extract were mixed together for 30 min).

Solvent exteracts	*Imbibryum* sp.	*Trichostomum* sp.	*Barbula convoluta*	*Fissidens* sp.	*Splachnobryum obtusum*	*Funaria hygrometrica*	*Didymodon* sp.	*Barbula* sp.
Ethanol	40%	45%	27%	36%	70%	42%	85%	55%
Methanol	92%	90%	94%	72%	18%	72%	27%	36%
Benzene	50%	44%	45%	45%	27%	54%	54%	45%
Petrolum ether	30%	20%	63%	80%	63%	36%	45%	18%
Acetone	27%	30%	36%	45%	63%	54%	45%	36%

In vivo experiments

Pre-inoculation experiment (bryophytes extracts treatment before virus infection)

Data in Table 6 showed that application of different bryophytes extracts before ZYMV infection led to variable inhibition ratios against the viral infectivity. The % of viral inhibition varied according to the bryophytes extracts and the time of application, where the highly inhibition rate was recorded after one day and the most potent solvent was aqueous methanolic extract of *Barbula convoluta, Imbibryum* sp. and *Trichostomum* sp. (Where they inhibited ZYMV symptoms by 87, 85 and 78, respectively). Morphological criteria of cucumber plants used in the pre- inoculated experiment were showed in Table 7 which revealed that the application of bryophytes extracts increase the growth of the plant (i.e. increase number of leaves, shoot length and fresh weight) more than the viral control.

Post-inoculation experiment (bryophytes extracts treatment after virus infection)

Data in Table 6 showed that application of different bryophytes extracts after ZYMV infection led to inhibition in the viral infectivity. Morphological criteria of plants subjected to post inoculation treatment were represented in Table 7. Generally, the obtained data revealed that foliar application of bryophytes extracts increase the growth of the plants more than the viral control.

Plant viruses are responsible for causing significant losses to the agricultural industry. The viruses are reported as being the second most damaging plant pathogen (Matthews,1992). The present study shows that the eight tested bryophytes are a potent inhibitor of ZYMV infection *in vitro* treatment. Results revealed that infection of cucumber plants with ZYMV caused symptoms mosaic, green blisters, maleformation, crinkle and leaf roll. These results are in according with that obtained by Al- Shahawan et al. (1995), Abdel- Shafi & Hussein (2012), Abdel-Shafi (2013) and Abdel-Shafi et al. (2013). Also, Tokuda et al. (1981) reported that some of the most affected crops by viral infection include potato, cucumber, tobacco, melons, strawberry, cabbage and radish. There is great demand for methods to reduce the damage caused by viruses and controlling viral diseases.

TABLE 5. Effect of 8 Bryophytesextracts on some morphological criteria of treated cucumber plants against ZYMV In vitro (Each value is the mean ten reading ± SD.).

Parameters	Ethanol	Methanol	Benzene	Petroleum ether	Acetone	Healthy	Viral
			Imbibryum sp.				
No. of leaves	5.9 ±a 0.6	6 ± a0.94	5.2 ±b 0.2	4.8 ±c 0.2	4.5 ± c 0.11	5.3 ±b 0.82	4.2 ± c 0.79
Shoot length	31.6 ±a 2.9	32.6 ±a 3.37	29.6 ±b 1.2	27.1 ±b 0.12	26.9 ± b2.1	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	5.4 ±a 0.4	5.62 ±a 0.5	5.00 ± a0.1	2.0 ±b 0.19	3.00 ± b 0.11	5.48 ± a0.56	2.01 ± b0.39
			Barbula convoluta				
No. of leaves	4.5 ±c 0.12	6.2 ±a 0.42	5.2 ±b 0.12	5.5 ±b 0.79	4.0 ± c 0.99	5.3 ±b 0.82	4.2 ± c 0.79
Shoot length	28.1 ±b 3.1	30.8 ±a 1.9	32.0 ±a 1.5	31.2 ±a 1.9	27.5 ± b 3.0	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	2.5 ±b 0.31	5.53 a± 0.42	5.50 ±a 0.34	5.1 ±a 0.49	2.7 ± b 0.16	5.48 ± a0.56	2.01 ± b0.39
			Trichostomum sp.				
No. of leaves	5.6 ±ab 0.30	5.8 ±ab 0.42	5.1 ±b 0.7	4.3 ±c 0.7	4.6 ± c 0.20	5.3 ±b 0.82	4.2 ± c 0.79
Shoot length	29.5 ±a 45	30.3 a± 0.67	30.5 ±a 0.4	26.9 ±b 2.9	28.5 ± b 2.2	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	5.25 ±a 0.12	5.19 ± a0.46	5.3 ±a 0.46	3.3 c± 0.29	2.9 ±b 16	5.48 ± a0.56	2.01 ± b0.39
			Barbula sp.				
No. of leaves	5.6 ± bc0.7	6.2 ±b 0.79	4.6 ±d 1.17	4.5 ±d 0.97	4.4 ± d 0.84	5.3 ±b 0.82	4.2 ± c 0.79
Shoot length	38.4 ±c 2.9	42.7 ±b 2.95	31.2 ±ef 6.5	34.0 ±de 3.83	30.4 ±f 5.3	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	7.07 ±b 1.03	8.33 ±a 0.67	98 ±c 1.11	4.79 ±c 1.34	5.15 ±c 0.79	5.48 ± a0.56	2.01 ± b0.39

TABLE 5. Cont.

Parameters	Ethanol	Methanol	Benzene	Petroleum ether	Acetone	Healthy	Viral
Funaria hygrometrica							
No. of leaves	5.4±c 1.27	6.9 ±b 0.74	5.1 ±cd 0.79	6.00 ±c 1.15	5.9 ±c 1.19	5.3 ±b 0.82	4.2 ±c 0.79
Shoot length	41.7±bc 5.44	49.3±a 6.02	41.2 ±bc 5.42	46.6 ±ab 6.41	38.4 ± c5.13	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	8.00±bc 1.56	9.5±a 1.43	8.3 ±ab 1.06	9.00 ±ab 0.25	7.00 ±c 2.16	5.48 ± a0.56	2.01 ± b0.39
Fissidens sp.							
No. of leaves	5.3±c 0.95	6.2±bc 1.03	5.7±bc 0.95	6.1±bc 0.99	5.8±bc 0.92	5.3 ±b 0.82	4.2 ±c 0.79
Shoot length	43.1±ab 3.72	47.15±a 6.27	38.6±b 4.48	39.6±b 5.09	4.18±b 5.63	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	8.7±b 1.64	11.9±a 2.88	8.9±b 0.74	8.4±b 1.17	9.3± b1.83	5.48 ± a0.56	2.01 ± b0.39
Splachnobryum obtusum							
No. of leaves	6.00±^{bc} 0.94	5.8± ^{bc}0.92	5.8±bc 1.48	6.5±bc 0.97	6.6±b 0.97	5.3±b 0.82	4.2±c 0.79
Shoot length	45.4±^{cd} 5.3	50.05±^{a} 3.93	44.7± cd3.62	49.3±ab 6	41.7±d 3.48	31.6±a 2.22	27.8±b 3.46
Fresh weight	9.4±^{b} 1.84	9.6±^{b} 1.71	11.9±a 2.51	9.76±b 2.27	8.66±b 1.65	5.48±a 0.56	2.01± b0.39
Didymodon sp.							
No. of leaves	5.8 ±d 0.79	7.1 ±bc 0.74	5.9 ±d 0.57	7.3 ±b 1.06	7.6 ±ab 0.7	5.3 ±b 0.82	4.2 ±c 0.79
Shoot length	33.4 ±c 4.6	40.75 ±b 2.52	33.7 ±c 3.56	41.85 ±b 4.38	32.8 ±c 5.2	31.6 ±a 2.2	27.8 ± b3.46
Fresh weight	5.8 ±e 0.66	8.29 ±bc 0.56	6.52 ±de 1.03	9.42 ±b 2.2	14.35 ±a 3.48	5.48 ± a0.56	2.01 ± b0.39

a, b,c, d means in the same raw with different superscript differ significantly ($P < 0.05$).

TABLE 6. Effect of bryophytes extracts on the infectivity of ZYMV (pre- inoculation and post- inoculation) on cucumber leaves at different time intervals.

Bryophytes / Extracts	*Barbula* sp.			*Didymodon* sp			*Funaria hygrometrica*			*Splachnobryum obtusum*			*Fissidens* sp.			*Barbula convoluta*			*Trichostomum* sp.			*Imbibryum* sp.		
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
Pre- inoculation																								
Ethanol	43%	40%	40%	70%	65%	72	40%	40%	35%	78%	60%	40%	34%	30%	25%	25%	25%	15%	42%	40%	35%	40%	40%	30%
Methanol	34%	30%	25%	25%	25%	60%	70%	65%	60%	15%	15%	10%	70%	65%	65%	87%	80%	70%	78%	70%	70%	85%	80%	80%
Benzene	43%	40%	40%	52%	50%	20%	52%	50%	45%	25%	20%	10%	42%	40%	35%	42%	40%	30%	42%	40%	35%	45%	40%	35%
Petrolum ether	15%	15%	15%	43%	40%	40%	34%	30%	30%	60%	55%	40%	67%	67%	60%	52%	50%	50%	20%	20%	20%	30%	30%	25%
Acetone	33%	30%	25%	43%	35%	40%	52%	45%	40%	62%	60%	55%	43%	40%	30%	34%	30%	30%	28%	25%	20%	27%	25%	20%
Post- inoculation																								
Ethanol	44%	30%	0	60%	40%	0	40%	35%	0	60%	40%	0	35%	30%	0	26%	20%	0	44%	30%	0	42%	40%	0
Methanol	35%	20%	0	25%	15%	0	70%	60%	0	17%	10%	0	80%	70%	0	80%	70%	0	70%	60%	0	80%	60%	0
Benzene	43%	28%	0	52%	40%	0	53%	40%	0	26%	20%	0	44%	40%	0	44%	30%	0	43%	35%	0	50%	40%	0
Petrolum ether	18%	10%	0	43%	30%	0	35%	30%	0	62%	50%	0	70%	60%	0	62%	50%	0	20%	10%	0	30%	25%	0
Acetone	34%	20%	0	44%	35%	0	53%	40%	0	62%	45%	0	44%	40%	0	35%	25%	0	30%	20%	0	25%	10%	0

TABLE 7. Effect of methanolic bryophytes extracts on some morphological criteria of treated cucumber seedling (24 h pre-inoculation and 24 h post-inoculation) against ZYMV (Each value is the mean twenty reading ± SD.).

Parameters \ Treatment	Healthy control	Viral control	*Imbibryum* sp.	Barbula convoluta	*Trichostomum* sp.
			24 h pre-inoculation		
No. of leaves	5.3±a0.82	4.2±b0.79	5.8±a0.63	5.5±a0.53	5.4±a0.52
Shoot length(cm)	31.6±ab2.22	27.8±c3.46	33.9±a3.51	31.4±ab2.12	29.2±bc3.19
Fresh weight (g)	5.48±a0.56	2.01±c0.39	5.53±a0.37	4.85±b0.74	4.51±b0.52
			24 h post-inoculation		
No. of leaves	5.3±a0.82	4.2±b0.79	6.5±a0.71	5.7±0b.48	5.5 ±b0.53
Shoot lengthcm)	31.6±ab2.22	27.8±c3.46	34.1±a2.42	31 ±b 2.26	30 ±bc 1.63
Fresh weight (g)	5.48±a0.56	2.01±c0.39	5.49±a0.38	5.17 ±a0.33	4.77 ±b 48

a, b,c, d means in the same raw with different superscript differ significantly ($P < 0.05$).

The results illustrated in Table 4 showed that the aqueous ethanol, methanol, benzene, petroleum ether and acetone extracts of eight mosses when mixed with the viral sap in equal volumes inhibited the viral symptoms compared to viral control. These results are in harmony with those Hillhouse (2003) who found that a large majority of the bryophytes species exhibiting considerable antiviral activity against PVX and as a result, the bryophytes have been identified as a new source of antiviral activity. The bryophytes were selected for this study because it was thought that the flavonoid and triterpenes contents might confer antiviral activity. The most potent bryophytes showed high antiviral activity in the initial screening were (*Imbibryum* sp., *Barbula convoluta* and *Trichostomum* sp.). Foliar application of the three bryophytes were tested against ZYMV before and after inoculation at different time intervals. The results of pre- inoculation experiments showed that application of bryophytes before ZYMV infection not only inhibit the virus but also increase the plant growth as compared with viral control. These results corroborated the findings of Asakawa et al. (2013) who reported that bryophytes show antiviral, plant growth regulatory and super oxide anion radical release.

The resultsin Table 6 also revealed that the application of bryophytes extract after mechanically inoculation of ZYMV at different time intervals inhibit the virus and increase the plant growth compared to viral control. Asakawa et al., (2013) showed that over several hundred new compounds have been isolated from the bryophytes and more than 40 new carbon skeletal terpenoids and aromatic compounds found in this class. Many of these compounds show antimicrobial, antifungal, antiviral, cytotoxic and anti- HIV. Until our knowledge this is the first time of studying the bryophytes to inhibit ZYMV.

Multiplication of virus particles in the infected plant cells alters primary and secondary biochemical compounds of cells such as chlorophyll, β-carotene, organic carbon, nitrogen, protein, phosphorus proteins, phenolic compounds and nucleic acids. External manifestations of disease symptoms are the results of altered host metabolism. The extent of crop loss is mainly associated with severity of visible symptoms (Chakraborty, 1993 and Charitha & Radha (2012). Lashin et al. (2015) reported that mosses plants contained numbers of secondary metabolites (flavonoids, tri terpens, tannins and saponines) in addition to carbohydrates and proteins. Asakawa (2008) reported that the bryophytes have biological activities due to previous compounds.

The present study showed that cucumber plants infected with ZYMV and treated with different mosses extracts contained photosynthetic pigments including chlorophyll (a and b) and carotenoids (Table 8). The highest contents of total photosynthetic pigments were detected in

infected cucumber plants which treated with methanol extracts of *Imbibryum* sp. while the lowest contents were detected in viral control plants. Actually decrease in photosynthetic rate of the infected leaves is often associated with development of the symptoms (Platt et al., 1979). Under greenhouse conditions the symptoms could be more clear and restricted. It should be independent of the virus and should reflect the host genetics. Virus replication in the infected plant cells exhibit some physiological and cytological changes such as chlorophyll, carotene, organic carbon, nitrogen, protein and phosphorus due to virus infection (Muqit et al., 2007). Various metabolites of host tissue were altered due to viral infection (Clover et al., 1999 and Hemida, 2002).

TABLE 8. Photosynthetic pigments of cucumber leaves (mg/g fresh weight).

Samples	Chlorophyll a	Chlorophyll b	Chlorophyll a + b	Carotenoid	Total pigment
Healthy	0.330± 0.002	0.210± 0.003	0.540± 0.073	0.003± 0.000	0.543± 0.083
Viral	0.200± 0.015	0.170± 0.001	0.370± 0.052	0.065± 0.001	0.435± 0.009
Ethanol	0.234± 0.051	0.299± 0.020	0.533± 0.016	0.009± 0.003	0.542± 0.004
Methanol	0.868± 0.039	0.240±0.336	1.108± 0.058	0.001± 0.000	1.109± 0.055
Benzene	0.340± 0.025	0.230± 0.010	0.570± 0.041	0.002± 0.000	0.572± 0.003
Petroleum ether	0.262± 0.081	0.289± 0.062	0.551± 0.006	0.004± 0.000	0.555± 0.047
Acetone	0.238± 0.009	0.304±0.029	0.541± 0.035	0.003± 0.000	0.544± 0.033

Physiological analysis

Photosynthetic pigments of bryophytes extracts and cucumber plants

The chlorophyll a, chlorophyll b and carotenoids, flavonoid, tannins, triterpenes, saponins and proteins of bryophytes *Imbibryum* sp., *Trichostomum* sp. and *Barbula convoluta* were shown (Lashin et al., 2015). The highest contents of total pigment and total phenols are detected in *Imbibryum* sp. Therefore, different solvent extracts of *Imbibryum* sp. were screened for antiviral activities. this results similar to that obtained by Wang et al. (2017).

The chlorophyll a, chlorophyll b and carotenoids of cucumber plants which infected by ZYMV and treated with different extracts of bryophyte contain was showing in Table 8. The highest contents of total pigment are detected in detected in cucumber plant which treated with methanol extract (1.109 mg/g fresh weight respectively) while the lowest contents of total pigment are detected in infected cucumber plant (0.435 mg/g fresh weight, viral infected cucumber plant).

Phenolic compounds of bryophytes extracts and cucumber plants

The tested mosses (*Imbibryum* sp., *Trichostomum* sp. and *Barbula convoluta*) contain number of phenolic compounds (e.g Flavinoids) including free and bound phenols (Lashin et al., 2015). The highest contents of free and bound phenols are detected in *Imbibryum* sp. (10.46 and 1.638 mg/g fresh weight for free phenols and bound phenols respectively).

The tested cucumber plants which infected by ZYMV and treated with different extracts of *Imbibryum* sp. contain a number of phenolic compounds including free and bound phenols as showing in Table 9. The highest contents of total phenols are detected in infected cucumber plant which treated with benzene extract (3.48 mg/g fresh weight) followed with methanol extract (3.19 mg/g fresh weight).

Total nitrogen and crude protein of cucumber plants

The total nitrogen and crude protein content of the tested cucumber plants which infected by ZYMV and treated with different extracts of *Imbibryum* sp. as showing in Table 10. The highest contents of crude protein and total nitrogen are detected in cucumber plant which treated with acetone (28.66 and 4.59 mg/g dry wt., respectively) while the lowest contents of crude protein and total nitrogen are detected in healthy cucumber plant (23.88 and 3.82, mg / g dry wt., respectively).

TABLE 9. Phenolic compounds of cucumber leaves (mg/ g fresh weight).

Samples	Free phenol	Bound phenol	Total phenol
Viral	1.32± 0.333	1.43± 0.501	2.75± 0.277
Healthy	1.09± 0.366	0.454± 0.136	1. 544± 0.633
Benzene	2.43± 0.556	1.05± 0.330	3.48± 0.530
Acetone	0.487± 0.052	0.244± 0.066	0.731± 0.100
Ethanol	1.13± 0.199	1.07± 0.730	2.2± 0.802
Methanol	2.118± 0.734	1.067± 0.291	3.185± 0.666
Petroleum ether	1.98± 0.651	0.756± 0.355	2.736± 0.501

TABLE 10. Total nitrogen and crude protein in cucumber leaves (mg/g dry weight).

Samples	Crude protein (mg/g dry weight)	Total nitrogen (mg /g dry weight)
Healthy control	23.88± 0.99	3.82 ± 0.16
Viral control	24.99 ± 1.04	3.99 ± 0.17
Ethanol extract	26.98 ± 1.12	4.32 ± 0.18
Methanol extract	26.60 ± 1.10	4.26 ± 0.18
Benzene extract	25.05 ± 1.04	4.01 ± 0.17
Petroleum ether extract	24.33 ± 1.01	3.89 ± 0.16
Acetone extract	28.66 ± 1.19	4.59 ± 0.19

The data are the means of three replicates ±SE.

The result presented in this work showed that cucumber plants infected with ZYMV and treated with different mosses extracts contained number of phenolic compounds including free and bound phenols. These results were in agreement with Kofalvi & Nassuth (1995), who reported a significant increase in phenols accumulation in wheat plants infected with the wheat streak mosaic potyvirus (WSMV) compared to the healthy controls. Phenolic compounds produced by plants are formed through phenyl propanoid metabolism. However, since free phenols can be cytotoxic in the cytoplasm, plants sequester these compounds in the vacuole or deposit them in or on the cell wall. Once the phenolic acids or cinnamyl alcohols reach the cell wall, they may be either esterified or linked to the cell wall polysaccharides or hemicelluloses, or be polymerized into lignin (Lam et al., 1992).

The accumulation of the phenolic compounds and their derivatives may be considered as a defense mechanism or as a hypersensitive reaction. The disease resistance response correlates with changes in cell biochemistry and physiology (Mohamed, 2008). Many studies showed that induced resistance through the accumulation of various phenolic compounds and activation of oxidative and key enzymes in phenyl propanoid and iso flavonoid pathways (Arfaoui et al., 2006). These results could give an explanation for the increase in phenolic compounds.

The result showed that cucumber plants infected with ZYMV and those treated with mosses extracts show high content of total nitrogen and crude protein as compared to healthy plants (Table 10). This result agreed with that obtained by Cheema et al. (2003), who showed that protein content in two soybean varieties increased with infection with soybean yellow mosaic virus. Rao et al. (1989) concluded that the increased protein content in virus infected plants was due to increased activity of RNA synthetase or RNA polymerase. The treated plants also show high protein content compared to viral control. This may be due to the formation of new antiviral protein. This agrees with that obtained by Abdel-Shafi (2005 and 2013).

Absence of toxicity of bryophytes (Barbula convoluta, Imbibryum sp. and Trichostomum sp.) in Wistar albino rats

Hematology parameters

The hematological profiles of the treated and control are represented in Table 11. All values were in the range of normal.

TABLE 11. Hematology parameters of SD rats treated orally with Bryophytes extracts for sub-acute toxicity (28 day).

	Dose (mg / kg body weight /day)						
	0	**250**	**500**	**250**	**500**	**250**	**500**
Substance	**Control**	***Imbibryum* sp.**		***Barbula convolute***		***Trichostomum* sp.**	
				Female			
RBC (x10 $^{6/\mu l}$)	7.67	8.48	8.39	6.95	7.04	6.80	7.81
WBC (x10 $^{3/\mu l}$)	8.9	6.4	3.0	12.0	4.9	5.8	7.3
(%)Hct	49.13	50.7	49.7	42.3	45.0	46.1	51.6
Hgb (g/dl)	14.43	15.6	15.2	13.0	13.7	14.0	15.3
MCV (fl)	64.03	59.8	59.2	60.8	63.9	67.7	66.1
MCH (pg)	18.83	18.4	18.2	18.7	19.5	20.6	19.6
MCHC (g/dl)	29.4	30.7	30.7	30.8	30.5	30.4	29.7
Platelets(x10$^{3/\mu l}$)	804	395	870	559	573	507	808
				Male			
RBC (x10 $^{6/\mu l}$)	7.00	7.35	7.70	6.56	7.79	7.50	6.96
WBC (x10 $^{3/\mu l}$)	4.7	9.4	7.7	8.3	8.9	6.1	4.8
(%)Hct	47.6	50.1	56.6	44.1	52.3	54.9	47.1
Hgb (g/dl)	13.8	14.6	16.0	12.7	15.1	15.4	13.2
MCV (fl)	68.0	68.1	73.4	67.2	67.1	73.2	67.7
MCH (pg)	19.7	19.8	20.7	19.4	19.4	20.5	19.0
MCHC (g/dl)	29.0	29.1	28.2	28.8	28.2	28.1	28.1
Platelets(x10$^{3/\mu l}$)	601	433	448	379	448	521	464

Values are mean for five rats/group ± SD.

WBC: White blood cell; RBC: Red blood cell; Hct: Hematocrit; Hgb: Hemoglobin concentration; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular concentration.

In this study these extracts of three mosses species were tested also for their acute and sub-acute oral toxicity in Wistar albino rats. The results showed that acute or sub-acute administration of bryophytes extracts is not toxic in male and female Wistar rats, suggesting a safety use by humans. The results found that administration of bryophytes extracts via the oral route up to a dose of 5000 mg/kg did not produce any mortality or alter behavioral patterns in the rats as compared to the control group. Weight gains in the treated male rats was not different from the control groups. According to the OECD guidelines (OECD, 2001a) for the testing of chemicals, the results of this acute toxicity study indicate that bryophytes extracts are fairly non-toxic. The LD_{50} of bryophytes extracts could not be calculated but it was assumed to be more than 5,000 mg/kg body weight/day. Substances with an LD50 between 5,000 and 15,000 mg/kg body weight/day are regarded as practically non-toxic (Loomis and Hayes, 1996).

The rats' relative internal organ weights were not altered by the bryophytes extracts (Fig. 7, 8 and 9). Furthermore, gross examination of the internal organs of all rats revealed no detectable abnormalities. In addition, the bryophytes extracts did not induce any damage to the internal organs as examined by blood parameters. Repeated administration of two doses (200 and 500 mg/kg body weight/day) for 28 days did not also produce evident signs of toxicity or pathogenicity on the body or organ weights indicating the absence of any specific organ toxicity since changes in body or organ weights are taken as indicators of toxicity (Andersen et al., 1999). Normal changes in body weight are considered to be indicators of food safety or lack of toxicity (Morita et al., 2011).

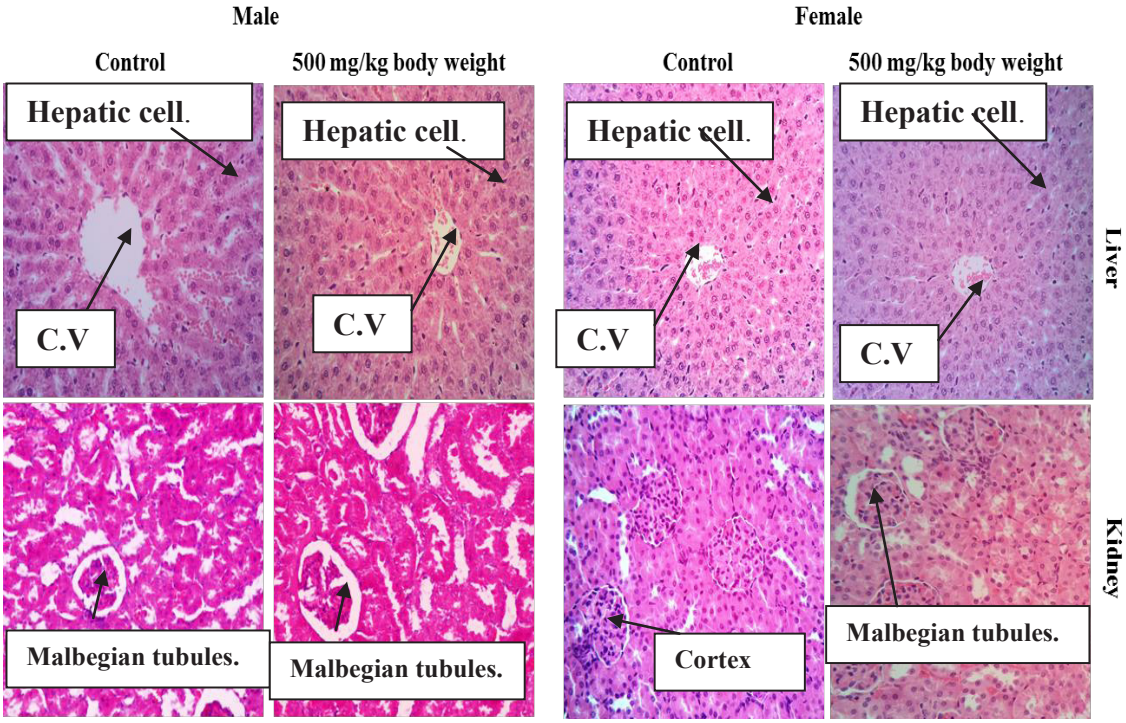


Fig.7. Representative microscopic findings in the liver and kidney of treated orally with *Imbibryum* sp. extracts for sub- acute toxicity (28 day).

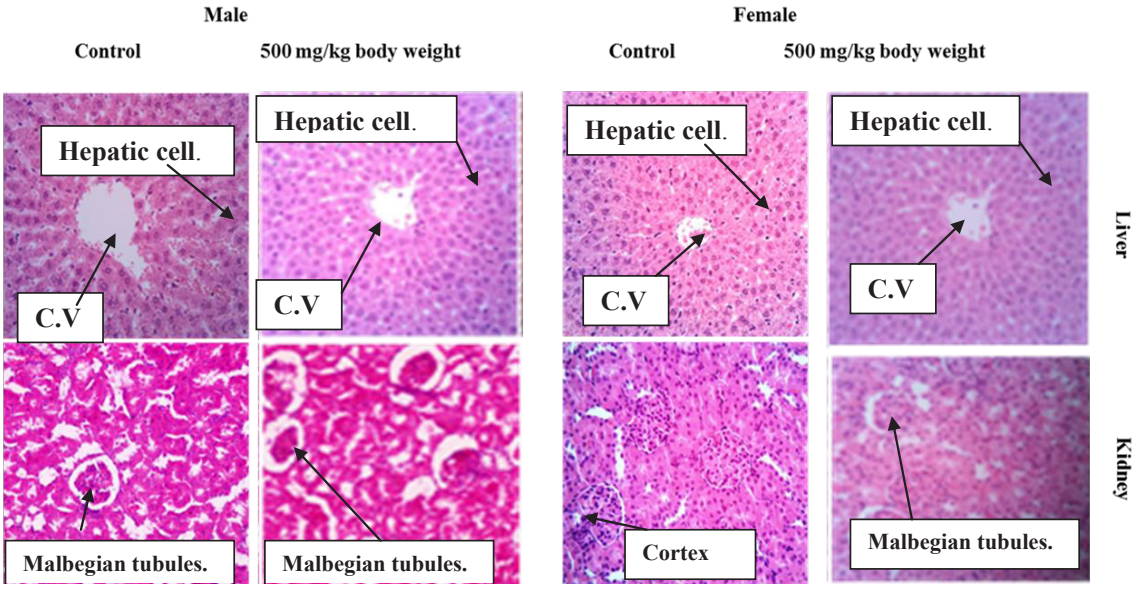


Fig. 8. Representative microscopic findings in the liver and kidney of treated orally with *Barbula convoluta* extracts for sub- acute toxicity (28 day).

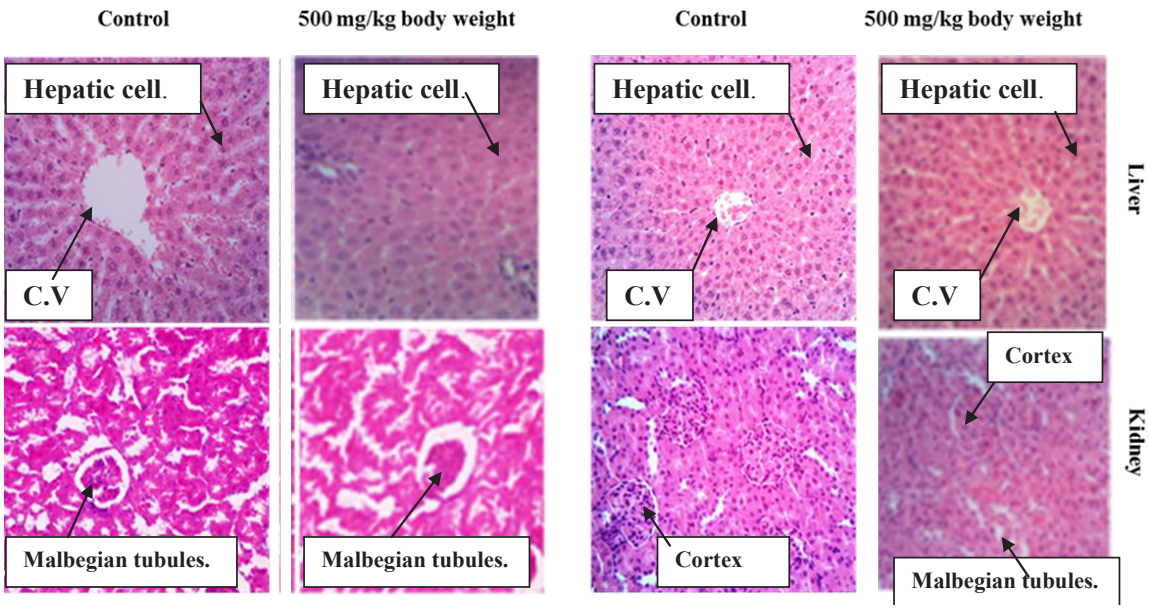


Fig. 9. Representative microscopic findings in the liver and kidney of treated orally with *Trichostomum* sp. extracts for sub- acute toxicity (28 day).

Conclusion

The highest degree of antibacterial and antiviral activities was shown by the aqueous methanolic extracts of bryophytes: *Imbibryum* sp., *Barbula convoluta and Trichostomum* sp., where such extract were found to contained a high percentage of active secondary metabolites as phenolic compounds with enhancement of growth of infected cucumber plants. Hence, the bryophytes have been identified as a new source of antibacterial and antiviral activities.

References

Abdel-Shafi, S. (2005) Biological studies on antiviral activities of some bacterial isolates *Ph.D. Thesis,* Department of Botany, Faculty of Science, Zagazig University, Egypt.

Abdel-Shafi, S. (2013) Preliminary studies on antibacterial and antiviral activities of five medicinal plants. *Journal of Plant Pathology and Microbiology,* **4,** 190.

Abdel-Shafi, S. and Hussein, Y. (2012) Biological control of Zucchini Yellow Mosaic Potyvirus (ZYMV) by *Bacillus firmus and Bacillus subtilis* in Squash plants. *The Egyptian Journal of Botany,* **52,** 1, 99-115.

Abdel- Shafi, S., Abdel- Gawd, S. and Sleem, E. (2013) Induction of Systemic resistance and enhanced enzyme activity by *Trichoderma* sp. Shmosa Tri (FJ 937359) in Squash against Zucchini Yellow Mosaic Virus (ZYMV). T*he Egyptian Journal of Botany, 3rd International Con.* pp.539- 558.

Allen, M. B. (1953) "*Experiments in Soil Bacteriology*", 1st ed. Burgess Pub. Co.

Al- Shahwan, I.M., Abdalla, O.A. and Al- Saleh, M.A. (1995) Response of green house-grown cucumber cultivars to an isolate of Zucchini Yellow Mosaic Virus (ZYMV). *Plant Disease,* **79** (9), 598- 601.

Andersen, H., Larsen, S., Spliid, H. and Christensen, N.D. (1999) Multivariate statistical analysis of organ weights in toxicity studies. *Toxicology,* **136,** 67–77.

Arfaoui, A., Sifi, B., Boudabous, A., El-Hadrami, I. and Cherif, M. (2006) Effect of *Rhizobium* isolates on isoflavinoids contents in chickpea plants infected with *Fusarium oxysporium* f. sp. ciceris. Phyto pathological Mediterranean.

Asakawa, Y. (1981) Biologically active substances obtained from bryophytes. *The Journal of the Hattori Botanical Laboratory*, **50**, 123–142.

Asakawa, Y. (1990) Biologically active substances from bryophytes. In: "*Bryophyte Development:*

Physiology and Biochemistry" Chopra, R.N., Bathla, S.C. (Ed.). Boston: CRC Press., p. 312.

Asakawa, Y. (2001) Recent advances in phyto chemistry of bryophytes- acetogenins, terpenoids and bis (bibenzyls) from selected Japanese, Taiwanese, New Zealand, Argentinean and European liverworts. *Phytochemistry,* **56**, 297–312.

Asakawa, Y. (2007) Biologically active compounds from bryophytes. *Pure and Applied Chemistry,* **79** (4), 557–580.

Asakawa, Y. (2008) Liverworts – potential source of medicinal compounds. *Current Pharmaceutical Design,* **14**, 3067–3088.

Asakawa, Y., Toyota, M., Tori, M. and Hashimoto, T. (2000) Chemical structures of macro cyclic bis (bibenzyls) isolated from liverworts (Hepaticae). *Spectroscopy,* **14**, 149-175.

Asakawa, Y., Ludwiczuk, A. and Nagashima, F. (2013) Chemical constituents of bryophytes: Bio- and chemical diversity, biological activity and chemosystematics. In: "*Progress in the Chemistry of Organic Natural Products*." Kinghorn, D.A., Falk, H., Kobayashi, J.. (Ed.), Springer, Vienna, pp. 1–796.

Assante- Duah, K. (2002) "*Public Health Risk Assessment for Human Exposure to Chemicals*". Springer. New York.

Balint, R., Plooy, I. and Steele, C. (1990) The nucleotide sequence of Zucchini Yellow Mosaic Potyvirus. Abstr.82 p. 1176. *Abstracts of the VIII the International Congress of Virology*, **8**, 84- 107.

Banerjee, R.D. and Sen, S.P. (1979) Antibiotic activity of bryophytes. *The Bryologist,* **82** (2), 141-153.

Basile, A., Giordano, S., Lopez-Saez, J.A. and Castaldo-Cobianchini, R. (1999) Antibacterial activity of pure flavonoids isolated from mosses. *Phytochemistry,* **52,** 1479–1482.

Benli, M., Yigit, N., Geven, F., Guney, K. and Bingol, U. (2008) Antimicrobial activity of endemic crataegus tan acetifolia (Lam.), pers and observation of inhibition effect on bacterial cells. *Biochemistry and Function,* **26**, 844- 851.

Blua, M.J. and Perring, T.M. (1989) Effect of Zucchini Yellow Mosaic Virus on development and yield of cantaloupe (*Cucumis melo*). *Plant Disease*, **73,** 317- 320.

Bodade, R.G., Borkar, P.S., Saiful, M.A. and Khobragade, C.N. (2008): *In vitro* screening of bryophytes for antimicrobial activity. *Journal of Medicinal Plants,* **7,** 23-28.

Chakraborty, S. (1993) Studies on viral diseases of cucurbits in Varanasi region. *Ph.D. Thesis*, Banaras Hindu University, Varanasi.

Charitha Devi, M. and Radha, Y. (2012) Induced biochemical changes in the CMV infected cucurbit plants. *Annals of Biological Research*, **3** (2), 863-867.

Chaudhary, B.L. and Kumar, P. (2011) Antibacterial activity and preliminary phytochemical screening of Epiphytic Moss *Stereophyllum Ligulatum Jaeg. International Journal of Pharma and Bio Sciences,* **2**, 4.

Cheema, S.S., Thiara, S.K. and Kang, S.S. (2003) Biochemical changes induced by soybean yellow mosaic virus in two soybean varieties. *Plant-Disease Research, Ludhiana, India,* **18,** 159-161.

Clover, G.R.G., Azam-Ali, S.N., Jaggard, K.W. and Smith, H.G. (1999) The effects of beet yellows virus on the growth and physiology of sugar beet (*Beta vulgaris*). *Plant Pathology*, **48**, 129-138.

Cole, J.O. and Parkers, C.R. (1946) Semi micro-Kjeldahl, procedurefor control laboratories."*Industrial and Engineering Chemistry Analytical Edition*", **18** , 61-62.

Desselberger, U. (1995) Molecular Epidemiology, In : "*Medical Virology: A Practical Approach*". Desselberger, U. (Ed.), pp. 173-190. Oxford University Press, New York.

Duncan, D. B. (1955) Multiple range and multiple F tests. *Biometrics*, **11,** 1–42.

Elibol, B., Ezer, T., Kara, R., Yuvalı Celik and Colak, E. (2011) Antifungal and antibacterial effects of some acrocarpic mosses. *African Journal of Biotechnology*, **10** (6), 986-989.

Flowers, S. (1973) "*Mosses Utah and the West*", Bringham Young University Press, Provo, Utah 84602.

Hemida, S.K. (2002) Effect of Bean Yellow Mosaic Virus on physiological parameters of *Vicia faba* and *Phaseolus vulgaris. International Journal of Agricultural and Biology*, **7** (2), 154-157.

Hillhouse, B.J. (2003) Screening of bi flavonoid compounds and British Columbian bryophytes for antiviral activity against Potato Virus X. MS. C. Faculty of Graduate Studies, British Columbia University.

Hojjati, S. (1976) Amino acid patterns of kidney beans grown under different S and K regimes. *Agronomy Journal,* **68,** 668 – 671.

Humason, G.L. (1979) "*Animal Tissue Techniques*",4th ed. W.H. Freem"r, uid Co., San Francisco.

Kandpal, V., Chaturvedi, P., Negi, K., Gupta, S. and Sharma, A. (2016) Evaluation of the antibiotic and biochemical potential of bryophytes from Kumaun Hills and Taral Belt of Himalayas. *International J. of Pharmacy and Pharmaceutical Sciences,* 8, 65-69.

Kothyari, B.P. (1997) Assay of bryophyte extracts for control of plant virus infections recent researches in ecology, *Environment and Pollution,* **11,** 461- 471.

Kofalvi, S.A. and Nassuth, A. (1995) Influence of wheat streak mosaic virus infection on phenylpropanoid metabolism and the accumulation of phenolics and lignin in wheat. P*hysiol. Mol. Plant Pathol.* **47**, 365-377.

Kozo, S.M., Handfi, J., Fujii, O., Sakanaka, K., Inuma, M., Ueki and Taniguchi, M. (1998) UK- 2A, B, C and D, novel antifungal antibiotic from *Streptomyces* Spp. 51702 III absolute configurations of an antifungal antibiotic UK- 2A and consideration of its conformation. *Journal of Antibiotics*, **151**, 113- 116.

Kumar, K., Singh, K.K., Asthana, A.K. and Nath, V. (1999) Ethno therapeutics of Bryophyte Plagiochasma appendiculatum among the Gaddi Tribes of Kangra Valley, Himachal Pradesh, *Indian Pharmaceutical Biology,* **37**, 1 - 4.

Lallyatt, C.I.K. (1977) Ultra structural changes in trifoliate leaves of bean (*Phaseolus vulgaris)* following inoculation of monili foliate leaves with (*Pseudomonas phseolica*). *Physiology and Plant Pathology,* **10,** 197-214.

Lam, T.B., Iiyama, T.K. and Stone, B.A. (1992)Changes in phenolic acids from internode walls of wheat and Phalaris during maturation. *Phytochemistry*, **31**, 2655-2658.

Lashin, G.M.A. (1990) Studies on bryoflora of Suez Canal Region, Egypt. *Msc. Thesis,* Botany Department, Faculty of Sciences, Zagazig University.

Lashin, G.M.A. (2011) Fine structures of some bryoflora spores from Saudi Arabia. *Egyptian Journal of Experimental Biology (Bot.),* **7** (1), 35-41.

Lashin, G.M.A., Abdel- Shafi, S., Hussein, Y., Osman, A. and Abdel- Monaem, Al. (2015) Efficient inhibition of pathogenic bacteria and potential toxicity by secondary metabolites of some bryophyte. *Egyptian Journal of Botany and Microbiology,* **3**, 475- 497.

Lisa, V., Boccardo, G., D' Aqostino, G., Dellavalle, G. and D' Aquillo, M. (1981) Characterization of a poty virus that causes Zucchini Yellow Mosaic Virus. Phyto Pathology, 71, 667- 672.

Loomis, T.A. and Hayes, A. W. (1996) "*Loomis's Essentials of Toxicology*". Academic Press, San Diego, CA.

Lopez-Saez, J.A. (1996) Biflavonoid differentiation in six *Bartramia* species (Bartramiaceae). *Plant Systematics and Evolution,* **203**, 83-89.

Lorimeres S.D. and Perry, N.B. (1994) Antifungal hydroxyl acetophenones from the New Zealand Liverwort, Plagiochila fasciculate), *Planta Med.* **60**, 386-387.

Matthews, R.E.F. (1992) "*Fundamental of Plant Virology*", pp-67-90. Academic Press. Inc. Harcourt Brace Jovanovich Publishers, San Diego, USA.

Meena, B., Marimuthu, T. and Velazhahan, R. (2001) Salicylic acid induces systemic resistance in groundnut against late leaf spot caused by *Cercosporidium personatum*. J*ournal of Mycology and Plant Pathology,* **31**, 139-145.

Metzener, H., Rau, H. and Senger, H. (1965) Untersuchungen zur synchronisier barkeit ein zeller-pigment- margel mutation von Chlorella. *Planta*, **65**, 186.

Ministry of Health and Welfare (1977) Canada Health Protection Branch: The testing of chemicals for carcinogenecity, mutagenecity and Teratogencity.

Mohamed, A.H. (2008) *In vitro* selection of soybean callus for wilt disease resistance *Ph.D. Thesis.* Faculty of Science Zagazig University, Egypt.

Morita, C., Nishida,T. and Ito, K. (2011) Biological toxicity of acid electrolyzed functional water: Effect of oral administration on mouse digestive tract and changes in body weight. *Archives of Oral Biology,* **56**, 359 – 366.

Muqit, A., Akanda, A.M. and Kader, K.A. (2007) Biochemical alteration of cellular components of ash gourd due to infection of three different viruses. *Int. J. Sustain. Crop Prod*. **2**(5), 40-42.

Murphy, F.A., Fauquet, C.M., Bishop, D.H.L., Ghabrial, S.A., Jarvis, A.W., Martelli, G.P., Mayo, M.A. and Summers, M.D. (1995) Virus taxonomy: Sixth Report of the International Committee on Taxonomy of Viruses. Wien Austria: Springer-Verlag, *Archives of Virology,* **10(Suppl.)**, 350- 354.

Nikolajeva, V., Liepina, L., Petrina, Z., Krumina, M.G. and Muiznieks, I. (2012) Antibacterial activity of extracts from some bryophytes. *Advances in Microbiology,* 2, 345-353.

Organisation of Economic Co-operation and Development (OECD) (2001a) The OECD Guideline for Testing of Chemical: 407 Repeated Dose Oral Toxicity-Rodent: 28- Day or 14-Day Study. OECD, Paris, France.

Perry, J. S. (1971) "*The Ovarian Cycle of Mammals*", Vol 13 (Oliver and Boyd, Edinburgh University Review Biology.

Platt, S.G., Henriques, F. and Rand, L. (1979) Effects of virus infection on the chlorophyll content, photosynthetic rate and carbon metabolism of *Tolmiea menziesii. Physiological and Plant Pathology Journal,* **16**,351–365.

Rao, G., Ghosal, M. and Shukla, K. (1989) Comparative study of carbohydrate and protein content of Radish Mosaic Virus infected, inhibitor treated and healthy radish plants. *Indian Journal of Virology,* **5**, 123-126.

Saeed, S., Naim, A. and Tariq, B. (2007) A study on prevalence of multidrug resistant Gram negative bacteria. *International Journal of Biology and Biotechnology,* **4**, 71-74.

Saritas, Y., Sonwa, M.M., Iznaguen, H., Konig, W.A., Muhle, H. and Mues, R. (2001) Volatile constituents in mosses (Musci).*Phytochemistry,* **57**, 443-457.

Singh, B. and Bhat, T.K. (2003) Potential therapeutic application of some anti nutritional plant secondary metabolites. *Journal of Agricultural and Food Chemistry,* **51**, 5579-5597.

Sitohy, M.Z., Mahgoub, S.A. and Osman, A.O. (2012) *In vitro* and *in situ* antimicrobial action and mechanism of glycinin and its basic subunit. *International Journal of Food Microbiology,* **154**, 19- 29.

Smith, D.K. (1994) In Crum H and Eckel pm. "*The Moss Flora of Mexico*", pp.427-442. Part 1. The New York Botanical Garden, New York.

Snell, F.D. and Snell, C.T. (1953) "*Colorimetric Methods of Analysis Including Some Turbid Metric and Nephelometric Methods*". D.van. Nostrend Comp. Inc. Princeton, New Jersy, Toronto, New York and London,101-111, p.666.

Sabovljević, M.S., Sabovljevic, A.D., Ikram, N.K.K., Peramuna, A., Bae, H. and Simonsen, H.T. (2016) Bryophytes –an emerging source for herbal remedies and chemical production. *Plant Genetic Resources*, 1–14.

Tedela, P.O., Adebiyi, A.O. and Aremu, A. (2014) *In vitro* antibacterial activity of two mosses: calymperes erosum C. Mull and Bryum coronatum Schwaegr from South-Western Nigeria. *Journal of Biology and Life Science*, **5**, 77-84.

Terry, T.M. (2007) "*Flora of North America*". Bryophyta. Part 1, Volume 27. Mosses. Fascicle. Funariaceae. Oxford University press, Inc. Oxford.

Tokudo, T., Ikedo, S., Kubota, Y., Takagi, T. and Kokagi, Y. (1981) "*Control of Plant- Virus Diseases*". US patent No. 4, 269- 857.

Wijk, R., Van Der, Margadent, W. D. and Florschutz, P. A. (1992) "*Index Muscorum*", Vols. 3. Regnum Vegetabile 33, Utretcht, Netherlands: Kermink en Zoon.

Webster's, P.J., Magana, V.O., Palmer, T.N., Shukla, J., Tomas, R.A., Yanai, M. and Yasunari, T. (1998) Monsoons: Processes, predictability, and the prospects for prediction . *Journal of Geophysical, Res.* **103** (c7) 14451-14510.

Wang, X., Cao, J., Dai, X., Xiao, J. Wu, Y. and Wang, Q. (2017) Total flavonoid concentrations of bryophytes from *Tianmu Mountain*, Zhejiang Province (China): Phylogeny and ecological factors. *PLOS Journal*. 1-10.

Yarwood, C. E. (1955) Mechanical transmission of apple mosaic virus. Hilgardia, **23**, 613 – 628.

Yount, N.Y. and Yeaman, M.R. (2005) Immune continuum: perspectives in antimicrobial peptide mechanisms of action and resistance. *Protein and Peptide Letters,* **12**, 49–67.

Zinsmeister, H.D. and Mues, R. (1987) Mosses as reservoir remarkable Sekundaerer. *Ingredients GIT Mag. Lab.* **31**, 499- 512.

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تقدير النشاط الضد بكتيرى والضد فيروسى لبعض الحزازيات

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هذه الدراسة تعين النشاط الضد بكتيرى والضد فيروسى لبعض مستخلصات الحزازيات. البكتريا الممرضة وهى ليستريا مونوسيتوجن، ايشرشيا كولاى، باسيليس سيريس و سيدوموناس اريجونوزا وتم تثبيطها بواسطة مستخلص الميثانول المائى للحزازيات الاتية *Imbibryum* sp., *Barbula convoluta* and *Trichostomum* sp وقد وجد ان خلط مستخلص*Imbibryum* sp مع المضاد الحيوى تتراسيكلين له تأثير تعاونى فى تثبيط بكتريا سيدوموناس اريجونوزا بينما خلط مستخلص*Trichostomum* sp مع تتراسيكلين له تأثير سلبى على تثبيط سيدوموناس اريجونوزا. وقد اوضح الفحص باستخدام الميكرسكوب الالكترونى الماسح ان بكتريا سيدوموناس اريجونوزا المعاملة بمستخلص الميثانول المائى ل *Barbula convolute* و *Imbibryum* sp و *Trichostomum* sp. احيطت بغلاف خارجى مقارنة بالبكتريا الكنترول الغير معاملة بمستخلص الحزازيات وتشوهت و اظهرت علامات عدم انتظام و تجعد السطح الخارجى و التصاق و تجمع الخلايا البكتيرية المحطمة. معظم الحزازيات المختبرة اظهرت نشاط ضد فيروس ZYMV وبناء على ذلك تعتبر الحزازيات مصدر جديد لتثبيط الفيروس النباتى. مستخلص الميثانول المائى للحزازيات الاتية *Barbula convoluta* *Imbibryum* sp و *Trichostomum* sp ثبطت ZYMV بنسبة 92،94 ،90%على التوالى. قد وجد ان نباتات الخيار التى تم حقنها ب ZYMV ومعاملتها بمستخلص البنزين ل *Imbibryum* sp ادى إلى زيادة المركبات الفينولية(mg/g fresh wt 3.48) يليه مستخلص الميثانول (mg/g fresh wt 3.19). مستخلصات الحزازيات ليس لها تأثير سام على فئران الوستر البينو.