Research Article

Evaluation of Pharmacognostic Features and Antimicrobial Activities of *Dysphania botrys* L.

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Abstract | *Dysphania botrys* L. is an annual herbaceous plant and has been utilized for the treatment of different ailments like asthma, cold, influenza, head ach, liver and digestive problems and healing of wounds. The aim of the study was to explore phytochemical constituents of methanolic crude extract (MCE) and various solvent fractions and *in-vitro* antimicrobial activities of whole plant of *D. botrys*. Qualitative phytochemical screening of MCE and solvent fractions of *D. botrys* showed the presence of alkaloids, phenols, flavonoids, saponins, tannins and sterols, however in n-hexane fraction (HxF) only flavonoids and saponins were detected. In quantitative analysis, among all the solvents, ethyl acetate fraction (EAF) had highest amount of phenol (27.4 mg/g), flavonoids (15.5 mg/g) and alkaloids (3.14 mg/g), while MCE displayed maximum amount of saponins (34.3 mg/g). In the proximate analysis, nitrogen freed extract was present in higher amount (38.45±0.83 %) followed by protein (30.26±0.72 %) while crude fibers were found least in amount (1.43±0.53 %). Among different minerals, reasonable amount of calcium (3268±0.53 µg/g), potassium (2873±0.71 µg/g), sodium (591±0.23 µg/g) and iron (223 ± 0.46 µg/g) were found while no cadmium and chromium was detected. MCE and EAF displayed considerable antibacterial activity against *Xanthomonas campestris* and *Pseudomonas aeruginosa* causing 12.6±0.54 mm and 20.6±0.53 mm zone of inhibition, respectively which was analogous to that of cefixime, used as standard drug. In case of antifungal activity MCE hindered the growth of *Fusarium oxysporum* effectively, causing 19.3±0.41 mm zone of inhibition, while the activity of other solvent was moderate.

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Introduction

Dysphania botrys L. synonym *Chenopodium botrys* L. belongs to family Amaranthaceae, having the English names sticky goosefoot, Ambrosia, Jerusalem oak and feather geranium. Previously *D. botrys* was placed in the genus Chenopodium, but due to recent taxonomic and phylogentic investigations, it was shifted to a separate genus, Dysphania (Clemants and Mosyakin, 2003).

D. botrys is native to Asia and Europe found in Pakistan, India, north Europe, Turkey Cyprus, Africa, Australia and North and South America (Seidemann, 2005). It is natural growing wild plant, traditionally used by the rural and endemic inhabitants of different regions of Pakistan for the curing of asthma, cough, wounds, fever, pain, liver, respiratory, urinary and gastric complaints (Khare, 2007; Hazart et al., 2011; Bano et al., 2014). In some areas of Kohistan, young and fresh leaves are used as an antiseptic and for wounds healing (Hazart et al., 2011). In Indian folk medicine, extract of *D. botrys* is well-known for urinary, digestive, respiratory, liver and stomach disorders

(Khare, 2007). *D. botrys* have characteristic odor due to presence of sesquiterpenes and monoterpenes (Kletter and Krichbaum, 2001). Secondary metabolites like alkaloids, flavonoids, phenols, terpenoids and ascaridole are present in varying amount depending upon its origin and habitat. Various studies carried out on contents of flavonoids in *D. botrys*, led to the separation of flavonoids which include; quercetins, chrysoeriol, hispidulin, flavones, 7-methyleupatulin, 5-methylsalvigenin, salvigenin, jaceosidin and sinensetin (Kletter and Krichbaum, 2001). Among alkaloids, betaine is present in prominent amount in all parts of plant and has been isolated (Khyalibova, 1968; Rustembekova et al., 1973).

In recent decades, the irrational utilization of antimicrobial drugs has been increased which lead to the growth and propagation of multidrug resistant types of pathogenic microbes (Aibinue et al., 2003; WHO, 2005). So, it is necessary to focus on extracting compounds from plants having significant antimicrobial activities. It is well established that certain chemicals, produced by plants for their own defense, have antimicrobial properties and are lethal for bacteria and fungi (Harborne, 1988). Thus, herbal crude extracts, various solvent fractions and the extracted compounds offer efficient source for the synthesis of antimicrobial drugs (Linton, 1983). Keeping in mind the medicinal significance of *D. botrys*, it was felt essential to evaluate active metabolites, proximate and mineral composition of whole plant of *D. botrys* along with antibacterial and antifungal activities, which may further provide scientific information to the scientific community.

Materials and Methods

Plant collection and identification

The plant material of *D. botrys* was collected from various part of District Swat, Khyber Pakhtunkhwa (KPK), particularly from marginal areas of river Swat. The plant was identified by plant taxonomist and was deposited in herbarium of University of Swat, having voucher number Swat 000411, for future reference.

Extraction of plant material

Plant material was cleaned with tap water and then dried out in shade at normal temperature for seventeen to twenty days time period. The dried plant material was minced in a Willy mills, then mixed and extracted three times with 80 % methanol for 72 hours at room temperature. It was filtered through Whatman's No.1 filter paper and then the combined filtrates were concentrated at 40-45 °C using rotary vacuum evaporator (Buchi, Switzerland). The final residue formed (790 g) was the methanolic crude extract (MCE). An amount of 490 g of the MCE was then subjected to further fractionation by utilizing n-hexane (3 x 1000 ml), dichloromethane (3 × 1000 ml) and ethyl acetate (3 × 1000 ml) solvents.

The crude extract and the three solvent fractions were used for phytochemical analysis and *in-vitro* antibacterial and antifungal activities.

Phytochemical investigation

Qualitative analysis of phytochemical: For qualitative analysis the following standard test were used.

Test for crude alkaloids: Alkaloids were detected in the plant extract by using Mayer's test. Dried plant extract of 50 mg and 10 ml dilute HCl was mixed through regular stirring and was then filtered. Two drops of Mayer's reagent were mixed to solution present in test tube. Formation of creamy or white color precipitate showed alkaloid presence (positive). Mayer's reagent employed in the test was of commercially grade (Tiwari et al., 2011).

Test for saponins: Saponins were detected by using Frothing test. Plant extract of 50 mg was diluted with distilled water up to 20 mL. Then the solution was poured in graduated cylinder and shake for 15 minutes. Formation of layer of foam indicated the presence of saponins (Kokate, 1999).

Test for phenols: Plant samples of 500 mg were mixed with 5 ml of distal water. Aqueous filtrate of each solvent was mixed with ferric chloride 2ml (5%) solution inside test tube. Formation of green color designated existence of phenols (Sofowora, 1993).

Test for flavonoids: Flavonoids were detected by using Alkaline's reagent test. Plant extract and fractions were mixed with solution of sodium hydroxide, formation of yellowish (golden) color which becomes colorless by adding CH_3COOH dilute solution, indicated the presence of flavonoids.

Test for tannins: Ferric chloride test was used for tannins detection. 50 mg of each sample was mixed with in 20 ml of deionized water. Then few drops of ferric chloride solution (0.1 %) were added to each

sample. Appearance of blue or black color confirms tannins existence (Sofowora, 1993).

Test for sterols: Salkowski test was used for the confirmation of phytosterols. Chloroform having volume of 2 ml was mixed with plant extract of 3 mg in test tube. The 2 ml of concentrated H_2SO_4 was added to it. The formation of red color in chloroform layer after shaking the solution for 5 minutes indicated the presence of phyto-sterols (Tiwari et al., 2011).

Quantitative analysis of phytochemical

Determination of total phenol: Total phenol contents in the methanolic extract and its consequent fractions were examined according to the protocol of Khan et al. (2008). 10 mg extract was mixed with Folin-Denis reagent (5 ml) and 20 % sodium carbonate (10 ml). The solution was diluted by using distal water by a factor of hundred. Solution was filtered and kept at ambient temperature for 10 minutes. Spectronic 20 D (Milton Roy) was used for the calculation of extract absorbance at 770 nm against blank. The total phenol concentration in the plant crude extract and others fractions was examined by matching with tannic acid constructed standard curve.

Determination of total saponins: Total saponins constituents in the plant extract and derived fractions were determined by Khan et al. (2010) method. Test sample of 2 g was put in small beaker and then 50 ml of petroleum ether was mixed and warmed on water bath for 5 minutes up to 40 °C with usual shaking. The solution was filtered and twice repeated the process along with further ether (50 ml). It was further extracted on gentle heating with methanol (5×48 ml). Then on water bath layer of methanol was concentrated up to 25 ml after which 150 ml of dry acetone was mixed for saponins precipitation. It was filtered and dehydrated to a constant weight at 90-100 °C by using oven.

Determination of total flavonoids: To study the total amount of flavonoids, 10 gm of crude extract and its fractions were mixed with 80 % methanol (10 ml). It was then filtered through filter paper i.e. Whatman's No. 42 and then the filtrate was put in crucible. Then using water bath, it was evaporated and then weighed.

Determination of total alkaloids: To examine the total contents of alkaloids in the plant extract and its solvent fractions Khan et al. (2010) protocol was followed. 2 gm of each sample was defatted by dissolving in ether and warmed up to 40 °C with regular shaking for 5 minutes on water bath. The solution was then acidified by treating with 100 ml acetic acid (20%) in C_2H_5OH and kept for four hours. The final solution obtained was filtered through filter paper and treated with NH_4OH in order to increase its pH value up to 9, followed by precipitation.

Proximate composition: Whole plant of *D. botrys* was utilized for proximate composition to investigate moisture, inorganic matter (ash), crude protein, lipid, fibers and carbohydrates by employing protocol reported by AOAC (2005). Inorganic matter was determined by dry ashing procedure while the amount of moisture was analyzed by using oven at 105 °C. The contents of protein were examined as N × Factor (100/16: 6.25 or according to Kjedahl method while lipids were extracted in organic solvent through Soxhlet apparatus. The amount of crude fibers was measured through titrimetric method. The carbohydrate contents were determined by the difference between the weight of sample and the sum of its moisture content, inorganic matter, crude lipid, crude protein, and fiber as described previously. Percent digestible carbohydrate was calculated as = 100 - (% Moisture + % Crude fiber + % Inorganic matter + % Crude lipid + % Crude protein).

Minerals composition: In order to examine mineral composition of plant, 1 gm sample of whole plant was taken in conical flask and solution of 10 ml (67 %) HNO_3 was mixed with it. The solution was kept at room temperature for 24 h after which 4 ml (67 %) of $HClO_4$ was added with it. It was concentrated by heating on hot plate at 55 °C until the formation of solution having around 1 ml apparent volume. After cooling the solution, distilled water was added and then filtered through filter paper (Whatman # 42). After that, final solution of 100 ml was prepared by adding distilled water, which was used as stock solution (Saeed et al., 2010). The analysis of copper, chromium, cadmium, lead, zinc, iron and nickel was carried by employing atomic absorption spectrophotometer while that of calcium, sodium and potassium was performed by employing flame-photometer (Jinway PFP7, UK).

Antimicrobial activity

Strains and culture media: Antibacterial activities of CME and various solvent fractions of *D. botrys* was evaluated against gram-negative bacteria such

as *Klebsiella pneumonia*, *Peseudomonas aeruginosa*, *Escherichia coli*,*Xanthomonas campestris*,*Proteus vulgaris* and gram positive bacteria such as *Staphylococcus aureus*, *Clavibacter michiganesis* and *Bacillus subtilis*, followed by antifungal activities against *Mucar pirimis*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium solani* and *Fusarium oxysporum*. All the bacterial and fungal strains were obtained from Department of Microbiology Quaid-e-Azam University Islamabad, Pakistan. The identity and purity of microbial strains were confirmed by the Department of Biotechnology and Microbiology, University of Peshawar. Strains of bacteria were cultured, and kept at 38 °C (on agar slants) while the different colonies of fungi were inoculated and kept on PDA (potato dextrose agar) at temperature of 28-30 °C. Bacterial and fungal stock cultures were kept at 4 °C.

Disc diffusion method: Antibacterial and antifungal effect of plant extract was determined by disc diffusion method according to the protocol of Rios et al. (1988) and Mbaveng et al. (2008), respectively. Sterile disc was soaked with 20µl of 10 mg/ml of MCE and other fractions and then placed on the inoculated agar and PDA plates. Cefixime and clotrimazole was used as standard reference drugs for bacteria and fungi. After keeping bacteria and fungi at 37 °C for 24 h and 72 h, the zone of inhibition was noted in millimeters.

Results and Discussion

Qualitative and quantitative analysis of phytochemical

In the qualitative analysis, MCE displayed positive results for alkaloids, phenols, flavonoids, saponins, tannins and sterols while in HxF only phenols and flavonoids were detected. DCMF and EAF gave negative results for tannins and sterols, respectively (Table 1). The quantitative analysis of selected phytochemicals showed that EAF had highest amount of phenol (27.4 mg/g), alkaloids (3.14 mg/g) and flavonoids (15.5 mg/g), while saponins were found maximum in MCE (34.3 mg/g) followed by EAF (28.4 mg/g). HxF displayed least concentration of all the tested phytochemicals (Figure 1).

It is not rational to study the biological assays of herbal derivatives without studying their chemical composition. Active compounds that are extracted from the plants play very important role in their

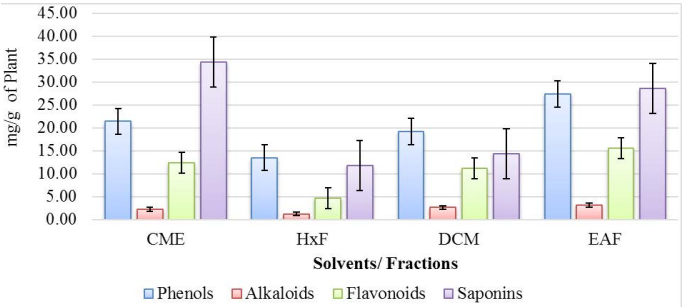

Figure 1: *Quantitative phytochemical analysis of methanolic crude extract and solvents fractions of D. botrys.*

Table 1: *Qualitative phytochemical analysis of methanolic crude extract and solvents fractions of D. botrys.*

Phyto-chemical	Name of test	MCE	HxF	DCMF	EAF
Alkaloids	Mayer's test	+	-	+	+
Phenols	Ferric chloride test	+	+	+	+
Flavonoids	Alkaline reagent test	+	+	+	+
Saponins	Frothing test	+	-	+	+
Tanins	Ferric chloride test	+	-	-	+
Sterols	Salkowski test	+	-	+	-

MCE: Methanolic crude extract, HxF: Hexane fraction, DCMF: Dichloromethane fraction and EAF: Ethyl acetate fraction; (+): detected; (-): not detected.

biological activities. In the current observation the presence of almost all bioactive metabolites in the crude extract and other solvents fractions authenticated the pharmacological potential of the plant. Our observation regarding phenolic contents was slightly greater than the amount reported by Ozer et al. (2016) in the same plant which might be due to its geographical location, nature of soil or environmental condition. Phenolic compounds present in the plant provide defense mechanism against reactive oxygen species, herbivores, insects and microorganisms (Vaya et al., 1997). In case of flavonoids, EAF displayed highest concentration (15.5 mg/g) which was in agreement with the observation of Panday and Gupta (2014) who reported 15.68 mg/g flavonoids contents in *C. album*. Flavonoids played an important role in a broad range of biological processes such as induction of cell apoptosis, reticence of cell-proliferation, enzyme inhibition, antioxidant and antibacterial activities (Middleton and Kandaswami, 1992; Cook and Samman, 1996). Maximum amount of alkaloids (3.14 mg/g) were present in the EAF, however this amount is relatively low as compared to other active metabolites. Alkaloids are chemical compounds, occurring naturally in nearly 20% of vascular plant species, most commonly in herbaceous dicots and

comparatively little in monocots and gymnosperms. (Hegnauer et al., 1988). These active compounds have role in plant protection against pathogens and herbivores. Isolated pure alkaloids and their derivatives are employed all over the globe for medicinal purposes due to their bactericidal, antispasmodic and analgesic properties (Harborne, 1988; Hartmann, 1991). Among all the tested metabolites, the high content of saponins (34.3 mg/g) indicated that this plant may be helpful in improving immunity system against pathogens, decreasing blood cholesterol level and the risk of intestinal cancer (Havsteen, 2002).

Proximate composition

The proximate composition analysis includes moisture, ash, protein, fiber, fat and nitrogen-free extract (carbohydrate) of whole plant of *D. botrys* as presented in Table 2. It was observed that the plant contains reasonable amount of life basic essential nutrients like carbohydrates (38.45%), protein (30.26%), fats (3.68%), while crude fibers (1.43%) were present in the least amount. The content of moisture was (7.45%) while amount of ash was (18.73%), represented the inorganic matter.

Table 2: *Proximate composition (%) of D. botrys whole plant.*

Content	Percent composition
Moisture	7.45±0.32
Ash	18.73±0.21
Fats	3.68±0.45
Protein	30.2 ±0.72
Fiber	1.43±0.53
Carbohydrates	38.4 ±0.83

All the values were taken as mean and standard error for each replicate (n=3).

The proximate composition analysis of whole plant of *D. botrys* displayed that it contains reasonable amount of carbohydrate (38.45%), suggested that the plant can be used as source of energy which play a key role in metabolism at the cellular level (Mensah et al., 2008). The amount of protein was 30.26%, which could contribute the daily protein requirement and also might act as good source of different amino acid having structural and functional role inside living organisms (NRC, 1975). The amount of fats contents (3.68%) was in the range as reported by earlier literature in other members of this genus (Ferreira et al., 2015). The content of crude fiber and moisture was low as compared to other plants. Low value of moisture in the whole plant is extremely useful in increasing shelf life of herbal drugs and decreases the chance of fungal and bacterial growth, which grows fast on substances having high moisture contents as compared to low moisture containing substances (Witthuhn et al., 2005). The value of ash (18.73%) designates the existence of inorganic substances in the plant extract and could be an excellent source of minerals. The content of ash is commonly considered as a measure of significance for evaluation of useful qualities of food (Hofman et al., 2002).

Mineral analysis

Mineral composition analysis of *D. botrys* plant showed reasonable amount of macronutrients like calcium, potassium and sodium having 3268 µg/g, 2673 µg/g and 591 µg/g concentrations respectively. Our results also indicated that it is a good source of iron and zinc, having 223 µg/g and 46.7 µg/g respectively. All the tested metals were present within the permissible limit and no chromium and cadmium were detected (Table 3).

Table 3: *Mineral composition of whole plant of D. botrys.*

Whole plant powder	Concentration (µg/g)
Ca	3268±0.53
K	2873±0.71
Na	591±0.23
Fe	223±0.46
Zn	46.7±0.32
Cu	8.3±0.48
Ni	1.2±0.16
Pb	0.4±0.13
Cr	ND
Cd	ND

All the values were taken as mean and standard error for each replicate (n=3).

The amount of different minerals analyzed in the whole plant of *D. botrys* may account in the ethno-pharmacological use of herbal extract for the cure of many diseases. The concentration range of different minerals in the plant may also helpful in employing its doses within the permissible limits. Among different minerals calcium was found in maximum amount, followed by potassium, sodium, iron, zinc, copper, nickel and lead while no cadmium and chromium were detected. All the tested macro and micro nutrients were present within the allowable limit (WHO, 1998). The high amount of calcium, potassium and

sodium in the whole plant indicated that it could be utilized for development and maintenance of healthy bones, function of muscles, production of enzymes, cell signaling, normal physiological functions and metabolic reactions inside the body (Aliyu et al., 2008). Cadmium and lead are the common phytotoxic heavy metals which disturb various biochemical and physiological processes, leading to inhibition of growth and cell death (Sandalio et al., 2001; Guo et al., 2009). These heavy metals have toxic effect on human health because these metals have no metabolic function and their intake in high amount may cause accumulation inside body, causing different noxious effects such as hepatic, cardiovascular and digestive disorders (Xu et al., 2009). It can be assumed on the basis of our observation that whole plant of *D. botrys* is an incredible resource of essential minerals which are necessary for regular physio-chemical performance of healthy body. The presence of all the minerals within the tolerable range and absence of toxic metals further authenticated that this plant could be utilized for pharmacological purposes and as a food supplement.

Antimicrobial activity

Antibacterial potential of plant methanolic extract and its subsequent fractions were evaluated, among which CME of plant exhibited effective antibacterial activity against *X. campestris* and *E. coli* causing 12.6±0.54 mm and 10.7±0.43 mm zone of inhibition, respectively. Among solvent fractions, EAF efficiently inhibited the growth of *P. aerugonosa* and *P. vulgaris,* causing 20.6±0.53 mm and 9.8±0.63 mm zone of inhibition, respectively. HxF showed least antibacterial activity against all the tested bacterial strains (Table 4). In case of antifungal activity, MCE exhibited considerable inhibiting effect against *F. oxysporum*, causing 19.3±0.41 mm zone of inhibition, while EAF noticeably reduced the growth of *F. solani* causing 12.5±0.53 mm zone of inhibition. Zone of inhibition was compared with that of reference drug clotrimazole, a standard antifungal drug. The overall growth inhibiting effect of tested solvent fractions were low to moderate against other fungal strains, while HxF showed no lethal effect on the growth of *A. flavus* and *A. niger* (Table 5).

In the present study, antibacterial and antifungal activities of crude extract and subsequent fractions of *D. botrys* against pathogenic bacteria such as *C. michiganesis, P. vulgaris* and *X. campestris* and fungal strains such as *A. flavus, A. niger, M. piriformis, F. solani* and *F. oxysporum* are reported for the first time. Our observations indicate that MCE and EAF were more effective than DCMF and HxF, in hampering the growth of some bacterial and fungal strains, however the overall effect against all the bacterial and fungal strains were low to moderate. One of the probable reasons of effective antimicrobial potential exhibited by MCE and EAF might be the presence of active secondary metabolites such as phenols, flavonoids, alkaloids, saponins, tannins and aromatic compounds (Bonjar et al., 2004). These compounds have the potential to restrain the growth of many pathogenic bacteria and fungi by attaching with proteins present at their surface, breaching peptide bonds and altering their biochemical composition or by inhibiting the intake of existing nutrient by microorganisms (Cowan, 1999).

Our results are also in accordance with that of Maksimovic et al. (2005), who stated that essential oil extracted from *C. botrys* exhibited inhibitory effect against different strains of bacteria like *Shigella flexneri*, *Salmonella enteridis, Klebsiella pneumonia, Sarcina lutea* and fungi like *Candida albicans* and *Aspergillus niger.* Similar observations are also reported by Foroghi et al. (2016) that its oil caused a decline in the growth of *E. coli* and *S. aureus.* The high antagonistic potential of MCE and EAF against some bacterial and fungal strains demonstrates that the plant might contain some effective metabolites which may possibly lead to isolation of novel antibiotics.

Conclusions and Recommendations

It can be concluded on the basis of our observation that whole plant of *D. botrys* is a rich source of nutrients and active metabolites, suggesting that this plant could be utilized in herbal medicine, as food supplement and can also be added in silage and fodder for domestic animals. Its extract may also be used for the treatment of different infectious diseases. Each value is the grand mean and standard error of three replicates. Dunnett t-test was used to compare the mean of standard as a control, and all other groups against it as a test. *= significant at the α> 0.05. NS= non-significant. Each value is the grand mean and standard error of three replicates. Dunnett t-test was used to compare the mean of standard as a control, and all other groups against it as a test. *= significant at the α> 0.05. NS= non-significant.

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Table 4: *Antibacterial activity of methanolic crude extract and solvent fractions of D. botrys.*

Bacterial strain	MCE (mm)	HxF (mm)	DCMF (mm)	EAF (mm)	*Standard (mm)
C. michiganesis	09.7±0.15*	06.4±0.61*	08.5±0.32*	08.7±0.42*	13.8±0.04
B. subtilis	11.8±0.26*	07.3±0.24*	13.7±0.06*	17.4±0.34*	22.6±0.15
P. Aerugonosa	15.3±0.18*	14.2±0.52*	17.5±0.13*	20.6±0.53*	24.7±0.32
K. pneumonia	13.6±0.41*	05.8±0.03*	08.7±0.51*	14.3±0.31*	26.3±0.12
S. aureus	10.5±0.31*	10.3±0.43*	08.6±0.63*	13.8±0.24*	18.5±0.21
E. coli	10.7±0.43*	05.3±0.51*	09.7±0.32*	07.5±0.41*	13.8±0.13
P. vulgaris	06.3±0.14*	07.2±0.31*	08.5±0.52*	09.8±0.63*	12.5±0.01
X. campestris	12.6±0.54*	06.8±0.42*	10.4±0.61*	07.4±0.71*	14.6±0.13

MCE: Methanolic crude extract; HxF: Hexane fraction; DCMF: Dichloromethane fraction and EAF: Ethyl acetate fraction; Standard: Cefixime.

Table 5: *Antifungal activity of methanolic crude extract and solvent fractions of D. botrys.*

Fungal strains	MCE (mm)	HxF (mm)	DCMF (mm)	EAF (mm)	*Standard (mm)
A.flavus	03.7±0.35*	00.00±0.00*	03.2±0.71*	07.3±0.42*	15.6±0.14
A. niger	04.2±0.13*	00.00±0.00*	03.5±0.63*	06.8±0.35*	18.3±0.41
M.piriformis	13.5±0.21*	07.8±0.41*	05.3±0.32*	12.4±0.02*	22.4±0.23
F. solani	09.5±0.51*	06.2±0.18*	06.4±0.12*	12.5±0.53*	14.7±0.36
F. oxysporum	19.3±0.41*	10.8±0.13*	13.6±0.71*	18.4±0.33*	24.8±0.15

MCE: Methanolic crude extract; HxF: Hexane fraction; DCMF: Dichloromethane fraction and EAF: Ethyl acetate fraction; Standard: Clotrimazol.

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Novelty Statement

The phytochemical and antimicrobial activities will be provided the way for herbal pharmaceutical industries to find the new and natural antibacterial and antifungal drugs which have no side effects on human body.

Author's Contribution

Dr. Asad Jan has supervised Mr. Muhammad Naeem Khan in his Ph. D program and this article is a portion of his research project.

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