Antibacterial and phytochemical analyses of *Dryopteris expansa* (C. Presl) Fraser-Jenk. & Jermy

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Abstract

Bacterial resistance against antibiotics has become a serious health issue in clinical practice, thus natural products can be use as additional drug. This is an alternative approach that could solve this problem. Therefore, the current study was conducted to thoroughly investigate the enrichment of phytochemicals and antibacterial potential of *D. expansa.*

The fronds samples of *D. expansa* prepared in different solvents (Chloroform, ethyl acetate, methyl alcohol, N-hexane, distill water) were used against MDR bacterial strains and phytochemical analyses.

The analyses of data revealed that *D. expansa* was enriched with flavonoids, alkaloids, saponins, quinines, tannins, glycosides, carbohydrates, carotenoids, terpenoids, phlobatanins, phenols, and coumarins while quantitatively this plant has a significantly higher content of phenols (994.42 µM/g) followed by sugar contents (740.99 µM/ml), ascorbic acid (425.60 mM/g), flavonoids (243.12 mg/g), anthocyanin (227.15 µM/g) and proline (0.23 µM/g). On the other hand, the organic extracts of *D. expansa* were highly active against all bacterial strains while hydric extract was inactive against selected bacteria. Specifically, *D. expansa* was highly active against *S. aureus* in all organic extracts (methyl alcohol= 18±1.52, ethyl acetate= 17±0.57, chloroform and n-hexane= 16.33±0.33) followed by *K. pneumonia* (n-hexane= 14.33±1.85, chloroform= 13.66±0.88, ethyl acetate= 12.66±1.45) and *E. coli* (chloroform= 14 ±0.57, methyl alcohol=

13.66±0.33, ethyl acetate= 12.33±0.33), while *P. aeruginosa* show resistant against all extracts except chloroform extract with 13±0.57 zone of inhibition.

Based on current findings it is concluded that *D. expansa* is enriched in many useful phytochemicals that could be use as a supplement with other traditionally used antibiotics.

Keywords: Phytochemical analysis, antibacterial activity, MDR, pteridophytes, *D. expansa*, fronds.

Introduction

The appearance and multiplication of drug resistant microbial strains with new resistance mechanisms exert pressure on the management of frequent infections. Most challenging and dangerous to whole globe is fast extension of multi and pan-resistant bacterial strains also known as super-bugs. The infections caused by these bacterial strains are impossible to cure with commonly available antibiotics [1]. In health care system antibiotic resistance is a major health issue and these MDR bacterial strains found everywhere. Excessive use of broad spectral antibiotics in aquaculture, animal husbandry and deficiency of fine antimicrobial stewardship are the major factors that contributes in spread of antibiotics resistance microbial strains [2].

Globally the major threat to public health is MDR bacterial strains. Massive economic loss in developing countries is due the treatment failure caused by multidrug resistance bacterial strains [3]. Cure of infectious diseases have complication due to multidrug resistance bacterial strain which results in towering loss of lives every year [4]. Major achievement in treatment of contagious diseases is represented by antibiotics innovation that greatly improves the hope and quality of life globally. Few years after the use of antibiotics MDR microbial strains are rapidly emerged and incessantly increases which creates major health concern all over the world [5]. In history of mankind and contemporary medicines no doubt antibiotic discovery is a milestone, they are crucial and life saving agent against various infectious disorders related to cancer chemotherapy and organ transplant, however since 1990s many antibiotics are discovered but loss their efficacy due the emergence of antibiotic resistance phenomenon [6].

According to WHO, drugs derive from traditional medicinal plants used by 80% of developing countries and WHO also listed the names 20,000 medicinal plant species which can be used as a potential source of new drugs [7]. Several countries develop rules for medicinal plants. More than 30,000 antimicrobial phytochemicals have been isolated from medicinal plants and more than 1340 plants with distinct antimicrobial potential have been analyzed [8]. Plants have

important phytochemicals with therapeutic efficacy throughout recorded history of humans. All civilizations have traditional medicines based on natural products and currently plants are considered as pool for detection of new drugs [9].

In comparison with synthetic drugs, plant based phytochemicals (phenols, alkaloids, coumarins, terpenes etc) are potent agents which can fight MDR microbial strains. These phytochemicals have various medicinal properties and can be used against the agents responsible for drug-resistance like bacterial cell communications, membrane proteins, efflux pump and biofilm [10]. Various plant secondary metabolites used in treatment of different conditions like caffeine used as stimulant, quinine as antimalarial drug, papaverine as phasphodiesterase inhibitor, scopolamine used against travel sickness and morphine used as analgesic [11]. Numerous medicinal phytochemicals from plants with multidirectional structures make them batter to cure fatal diseases and some of these already been used in novel drug discovery [12].

The role of pteridophytes (ferns) in traditional medicines is well known. Various systems of medicines effectively used pteridophytes in remedies like Unani system, Ayurvedic system and homeopathic system [13]. Pteridophytes develop many morphological characters and secondary metabolites to tolerate the harsh conditions on land environment. Some of these phytochemicals show medicinal potential thus can be used as powerful drug against pathogens [14]. Pteridophytes have verity of bioactive compounds like terpenoids, steroids, Flavanoids and phenolics etc, different isolated compounds and extracts of pteridophytes have verity of biological activities [15].

In general the literature survey shows the medicinal significance of pteridophytes, so keeping in view the importance of Pteridophytes, *Dryopteris expansa* belongs to family Dryopteridaceae was selected to determine the wide image of their phytoconstituents and antibacterial activity against selected multidrug resistant bacterial strains.

Materials and methods

Plant collection

Fern *D. expansa* was collected from District Mansehra and processed at the Department of Botany Hazara University Mansehra, KPK, Pakistan. First of all, fronds of the plant were air-dried and powdered by using a commonly used blender. 50g powdered samples were dissolved in 400ml of 5 different solvents (Chloroform, Methyl alcohol, N-hexane, Ethyl acetate, and Distilled water) and kept in a shaker for 7 days at room temperature (25±2°C). Then each sample

was filtered out using filter paper (F1001 grade) and subjected to a rotary evaporator for completely drying and concentrating samples, and stored at 4◦C till further analyses. All the sampling and experimental procedures were approved by the advance studies & research board (ASRB) and ethical committee of Hazara University, Mansehra, Pakistan.

Phytochemical analyses

Plant samples were prepared in different solvents for determining various phytochemicals; different phytochemicals were detected based on changes in the solvents' colors. For terpenoids, flavonoids, saponins, tannins, phenols, carbohydrates, and glycosides we followed the protocols published by [16], while alkaloids, Phlobatanins, coumarins, and carotenoids were analyzed by standard protocols mentioned in previous studies [17]. To quantify the phytochemicals in *D. expansa,* we used a spectrophotometer (UV 1900), by following the manufacturer's guidelines. Specifically, flavonoids and phenols [18], sugar content [19], anthocyanin [20], ascorbic acid [21], and prolines [22] were accordingly determined by following the modifications mentioned in the studies.

Antibacterial activity

Four bacterial MDR strains (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus,* and *Klebsiella pneumonia*) were used to determine the antibacterial potential of *D. expansa*. Dry extracts were dissolved in DMSO (Dimethyl Sulphoxide) in a proportion of 120mg/ml and the disc diffusion method was applied to determine the antibacterial effects of plant samples. Standard antibiotic levofloxacin (5µg) and DMSO were used as positive and negative control/blank respectively [23].

Statistics

Statistical analyses were performed by using software (statistix 8.1). All experiments were performed in triplicate, and the mean of replicates was noted. The ANOVA test was performed to observe significant differences among different treatments. A P-value ≤ 0.05 was considered significant.

Results and discussion

Phytochemical analysis

Qualitative phytochemical analysis

Table.1 Phytochemical constituents of *Dryopteris expansa* in different extracts

Tests	Chloroform	Ethyl acetate	Methyl alcohol	N-hexane	Distilled water
Flavonoids	+++	+++	-	+++	++
Carotenoids	+++	+++	+++	+++	-
Terpenoids	-	-	+++	++	+++
Phlobatanins	++	-	++	-	++
Alkaloids	++	+++	-	+++	++
Saponins	+++	-	+++	-	-
Quinines	-	-	++	++	++
Carbohydrates	++	-	++	-	++
Glycosides	++	-	++	-	++
Phenols	+++	-	+++	-	++
Tannins	+++	-	+++	-	-
Coumarins	-	+++	++	+++	-

Key = -, absent; ++, moderately present; +++, strongly present

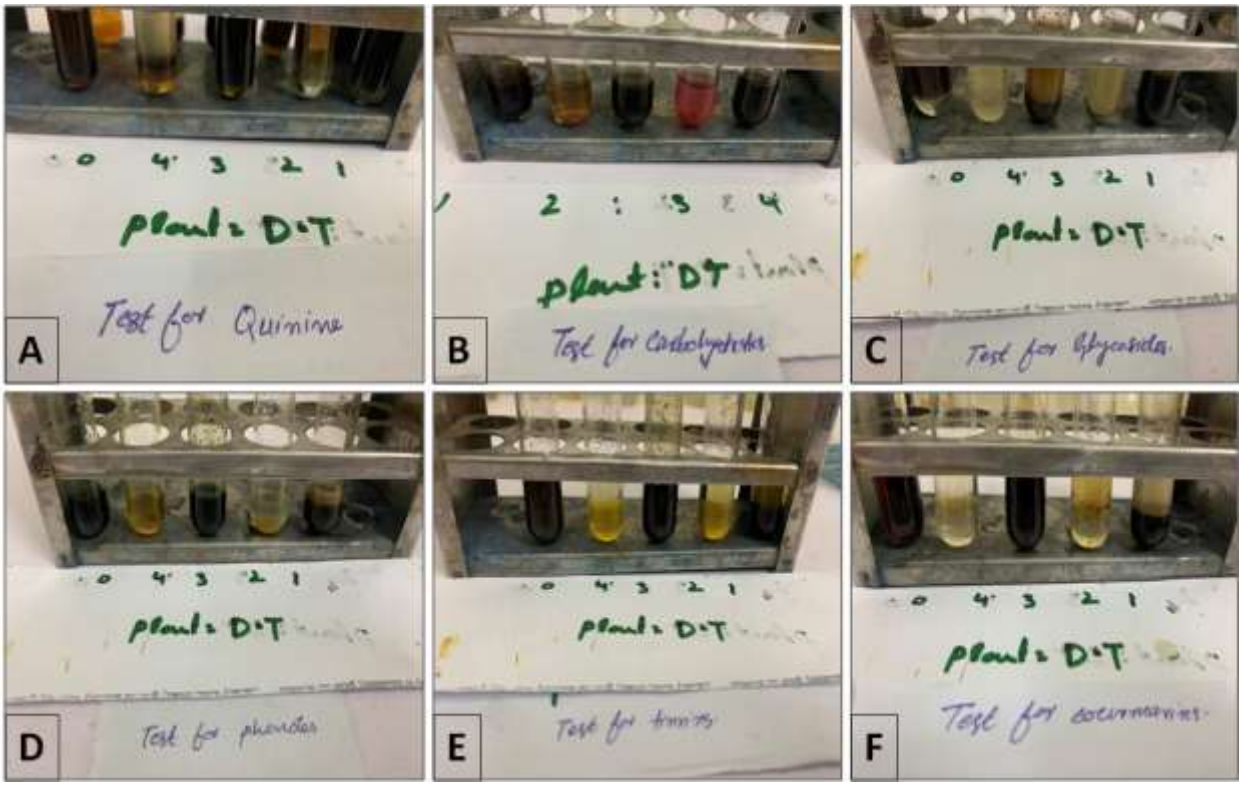

Figure.1 Qualitative phytochemical analysis of *D. expansa*, A=test for quinine, B= test for carbohydrates, C= test for glycosides, D= test for phenols, E= test for tannins, F= test for coumarins.

Table 1 figure 1 explains the qualitative phytochemical analysis of *D. expansa*. All the extracts were observed to have phytochemicals with few exceptions. Flavonoids, Carotenoids, saponins, phenol and tannins were strongly present in chloroform extract while Phlobatanins, carbohydrates, alkaloids and glycosides were moderately present in chloroform extract. Chloroform extract of *D. expansa* show the absence of terpenoides, quinines and coumarins. Strong presence of Carotenoids, terpenoids, saponins, phenols and tannins was shown by methyl alcohol extract while Phlobatanins, quinines, carbohydrates, glycosides and coumarins were moderately present in methyl alcohol extract. Flavonoids and alkaloids were absent from methyl alcohol extract. The samples prepared in methyl alcohol and chloroform showed good efficacy for the identification of phytochemicals, specifically, higher phytoconstituents were detected in the methyl alcohol followed by chloroform extract. Similar findings have been reported by [24], claiming that methyl alcohol is the best solvent for the detection of different classes of phytochemicals. previous study also show that Chloroform and methyl alcohol are the best solvents for identifying alkaloids, tannins, sugar, saponins, flavonoids, terpenoids, cardiac glycosides, phenolics, and anthraquinone [25]. In addition N-hexane has also been considered the best solvent for analyzing different classes of phytochemicals [26].

Strong existence of flavonoids, Carotenoids, alkaloids and coumarins was observed in ethyl acetate extract while other phytochemicals were absent from ethyl acetate extract of *D. expansa*. N-hexane extract was observed to show strong existing of flavonoids, Carotenoids, alkaloids and coumarins while terpenoids and quinines were moderately present and N-hexane extract of *D. expansa* show absence of Phlobatanins, saponins, carbohydrates, glycosides phenol and tannins. Moderate presence of phytochemicals (flavonoids, Phlobatanins, alkaloids, quinines, carbohydrates, glycosides and phenols) was observed in distill water extract. Only terpenoids was observed to have strong presence in distilled water extract, while Carotenoids, saponins, tannins and coumarins were absent from distilled water extract. We successfully identified flavonoids, alkaloids, saponins, tannins, glycosides, carotenoids, terpenoids, phlobatanins, phenols and coumarins compounds in *D. expansa*. Individually these compounds have medicinal

and commercial importance [27]. Presence of these bioactive compounds guaranteed that our plant (*D. expansa*) has potent medicinal characteristics.

Quantitative phytochemical analysis

Table.2 Quantitative phytochemical analysis of *D. expansa*

S.NO	Phytochemicals	Quantities (average ± standard error)	Units
1	Ascorbic Acid	425.60 ± 0.25	mM/g F.WT
2	Flavonoids	243.12 ± 40.14	Mg/g F.WT
3	Reducing Sugars	740.99 ± 21.79	uM/ml F.WT
4	Phenolics	994.42 ± 21.69	Mg/L F.WT
5	Prolines	0.23 ± 0.11	uM/g F.WT
6	Anthocyanins	227.15 ± 5.97	uM/g F.WT

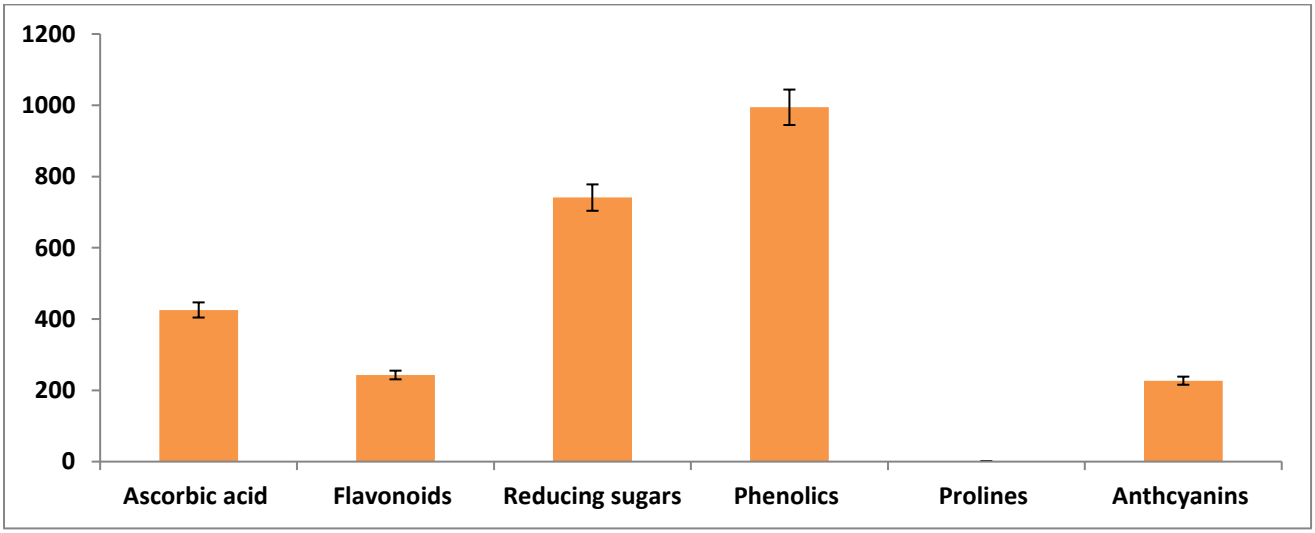

Figure.2 Graphical representation of quantitative phytochemicals of *D. expansa*.

Table.2 and figure.2 represents the quantification of various phytochemicals in *D. expansa*. Significantly higher quantities of phenolics (994.42 ± 21.69) and reducing sugars (740.99 ± 21.79) were observed in *D. expansa* followed by Ascorbic acid (425.60 ± 0.25). In contrast to these phytochemicals fewer amounts of flavonoids (243.12 ± 40.14) and anthocyanins (227.15 ± 5.97) were determined during spectrophotometry, while non-significant proline contents (0.23 ± 0.11) were observed in *D. expansa*.

Secondary metabolites (phytochemicals) help the plants to cope with adverse environmental conditions and also have medicinal importance for human [28]. Flavonoids have antimicrobial, anti-inflammatory, anti-carcinogenic and antioxidant [29]. Alkaloids are cardio protective, anti-inflammatory and anesthetic agents. Morphine is well known alkaloid used as strong analgesic drug [30]. Plants phenolic compounds have oxygen free radicals scavenging property; can be used against oxidation reactions in the body which prevent the development of cardiovascular disorders [31]. Similarly prolines in human body act against oxidative stress in different diseases [32]. Anthocyanins are medicinally most important phytochemicals can be used in different aliments such as cardiovascular, neurological, cancer, diabetes and gastric disorders [33].

Qualitative and quantitative phytochemical analysis of *D. expansa* shows the presence of different secondary metabolites which indicates the medicinal significance of this plant.

Antibacterial activity

Table.3 Antibacterial activity of *D. expansa*

Zone of inhibition (mm) ± Standard Error means

S.NO	strains	CH	EA	MA	NH	DW	LEVO
1	*S. aureus*	16±0.57	17±0.57	18±1.52	16.33±0.33	00±00	24.66±0.88
2	*K. pneumonia*	13.66±0.88	12.66±1.45	7.33±3.66	14.33±1.85	00±00	14±1.15
3	*E. coli*	14 ±0.57	12.33±0.33	13.66±0.33	7.66±3.92	00±00	20.33±0.88
4	*P. aeruginosa*	13±0.57	3±3.00	6.33±3.17	5.33±5.33	00±00	19±0.57

Key- CH= chloroform, EA= ethyl acetate, MA=methyl alcohol, NH= n-hexane, DW=distilled water, LEVO= Levofloxacin

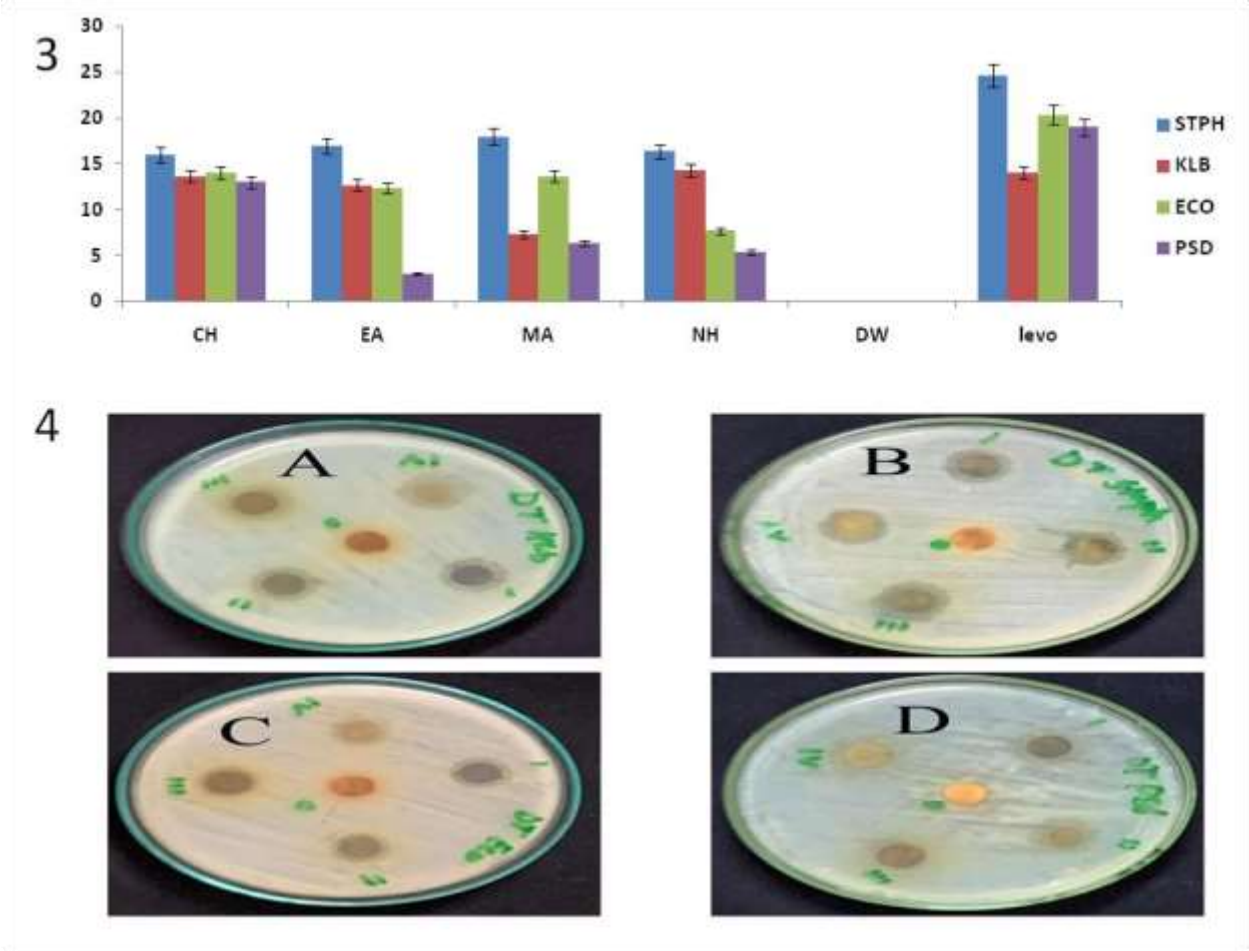

Figure 3. Graphical representation of antibacterial activity of *D. expansa*. (STPH= *S. aureus*, KLB= *K. pneumoniae*, ECO= *E. coli*, PSD= P. aeruginosa) CH= chloroform, EA= ethyl acetate, MA=methyl alcohol, NH= n-hexane, DW=distilled water, LEVO= Levofloxacin.

Figure 4. Pictures of antibacterial activity of *D. expansa* against selected bacterial strains (4A= activity against *K. pneumonia*, 4B= activity against *S. aureus*, 4C= activity against *E. coli*, 4D= activity against *P. aeruginosa*) key of tags on plates (DT= *D. expansa*, i= chloroform extract, ii= ethyl acetate extract, iii= methyl alcohol extract, IV= N-hexane extract, 0= distill water extract).

Figure 3 and table 3 showed the antibacterial activity of four extracts of *D. expansa* against four bacterial strains. All the extracts show resistant against all bacterial strains except distilled water extract which show no antibacterial potential. We assume that the antibacterial compounds in our plant extract are non-polar in nature and distill water can't dissolve them due this distill water extract of *D. expansa* is in active against any of selected bacteria. Results of the current study is

similar to findings of [34], they have observed the antibacterial potential of Marigold leaf extracts in oil and water. Both extracts did not show any inhibitory zone against selected bacterial strain p*seudomonus* spp, *Klebsiella* spp, *V. cholerae and S. aureus*. Similar result was also coded by [35], that water extract is unable to produce any zone of inhibition against *S. aureus and Pseudomonus* spp. Similarly the results of current study are in contrast with the findings of [36], they reported that the aqueous extract of rosemary was highly active against food pathogens and spoilage microorganism (*E. coli, B. aureus* and *S. aureus*).

Maximum zone of inhibition (18±1.52) was measured in methyl alcohol extract against *S. aureus* followed by ethyl acetate extract (17±0.57), while N-hexane and chloroform extracts was also effective against *S. aureus* with inhibitory zones (16.33±0.33) and (16±0.57) respectively (fig. 4B). N-hexane extract show maximum zone of inhibition against *K. pneumonia* which was recorded as (14.33±1.85), followed by ethyl acetate extract and chloroform extract with inhibitory zones (12.66±1.45) and (13.66±0.88) respectively, while minimum zone of inhibition was recorded (7.33±3.66) in methyl alcohol extract (fig. 4A). Highest resistance zone (14 ±0.57) was measured against *E. coli* in chloroform extract followed by methyl alcohol (13.66±0.33) and ethyl acetate extracts (12.33±0.33), while lowest inhibitory zone was observed in N-hexane extract (7.66±3.92) (fig. 4C). Similarly *D. expansa* was shown to be less effective against *P. aeruginosa*. Maximum zone of inhibition was recorded in chloroform extract (13±0.57), followed by methyl alcohol and N-hexane extract which was measured as (6.33±3.17) and (5.33±5.33) respectively, while ethyl acetate extract show non-significant inhibition (3±3.00) against *P. aeruginosa* (fig. 4D).

Control antibiotic discs levofloxacin (5µg) ware used which show significant resistance against all selected bacterial strains. Maximum zone of inhibition was calculated (24.66±0.88) against *S. aureus* followed by *E. coli* (14±1.15), similarly antibiotic disc was also effective against *K. pneumonia* with inhibitory zone (14±1.15), which show none significant difference when comparing with ethyl acetate, N-hexane and chloroform extracts. Control disc show resistance against *P. aeruginosa* with zone of inhibition (19±0.57), which show significant difference as comparing with other plants extracts.

Organic solvents extracts of *D. expansa* were highly active against all the selected bacteria we speculate that the antibacterial components of our plant are non-polar in nature and non-polar solvent dissolve them which in turn show antibacterial activity. Chloroform and ethyl acetate

extract shows significant activity against all selected bacterial strains. Our results are in conformity with the findings of [37] they observed that the ethyl acetate and chloroform extracts of *U. wallichiana* show remarkable activity against *P. aeruginosa* and *B. subtilis.* Our findings are in contrast with those coded by [38] they claimed that methanol extract show best antibacterial activity in comparison with chloroform and ethyl acetate plant extract. During the experiment we observed that *S. aeurus, K. pneumoniae, E. coli* are most susceptible strains to our organic solvents extracts while *P. aeruginosa* was most resistant strain to *D. expansa* extract. Similar findings are present in literature that *E. coli, S. aeurus* [39] *K. pneumoniae* [40] are most susceptible strains to plant extract. Regarding P. aeruginosa opposite findings are present in literature [41] they claimed that different plant extracts have bacteriostatic effects on *P. aeruginosa*. Findings of present study support the significance of *D. expansa* as a source of active phytochemicals for the treatment of diseases caused by *S. aeurus, K. pneumoniae, E. coli*. Based on these findings it is concluded that *D. expansa* is enriched in bioactive phytochemicals and their organic extracts are highly active against selected multidrug resistant bacterial strains. The phytoconstituents from this plant can be used to combat the diseases caused by bacterial strains used in the study which require further clinical validation.

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