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ANTIMICROBIAL ACTIVITY OF PHENOLIC AND FLAVONOIDS ENRICHED EXTRACTS OF *DOLOMIAEA MACROCEPHALA*-ASTERACEAE: A MEDICINAL PLANT FROM WESTERN KASHMIR HIMALAYAS

MUHAMMAD JAMIL AHMED*, GHULAM MURTAZA AND FAROOQ AHMED

Department of Botany, King Abdullah Campus, University of Azad Jammu and Kashmir (UAJK), Muzaffarabad-13100, Pakistan
**Corresponding author's email: jammumughal.bot86@gmail.com*

Abstract

The objective of current study was to investigate total phenolic, flavonoids and triterpenoids content in various solvent extracts from leaves and roots of *Dolomiaea macrocephala* and their inhibitory effect against pathogenic bacteria and fungi. Disc diffusion method was adopted to find out antibacterial and antifungal potential of various solvent extracts. Total phenolic, flavonoids and triterpenoids content were determined by the Folin–Ciocalteu, aluminium chloride colorimetric method and vanillin–glacial acetic acid method respectively. Their correlation with antimicrobial activities was statistically analyzed by Pearson's correlation coefficient method. The present study revealed highest phenolic and flavonoids content in methanol and aqueous extracts in both leaves and roots of *D. macrocephala*. The highest triterpenoid content was recorded in petroleum ether extract. Various solvents extract from leaves and roots showed significant bactericidal effect against both Gram Negative and Gram-Positive bacteria. However, only aqueous and methanol extracts of *D. macrocephala* exhibited remarkable zone of inhibition against post-harvest fungal pathogens. The Pearson's correlation revealed a significant positive correlation between total phenolic and flavonoids content in various solvent extracts in both leaves and roots with their antibacterial activity. The findings from present study revealed that antibacterial activities of different solvent extracts from leaves and roots were due to highest phenolic and flavonoids content. Thus the study insight the presences of major bioactive compounds in plant extract and showed their potential antimicrobial application against antibiotic resistant bacteria.

Key words: Phytochemical screening, Disc diffusion assay, Pearson correlation, Solvent extracts, Total phenolic.

Introduction

Plants have been used traditionally in health care system since the beginning of human civilization and the medicinal plants extract being used for sighting bioactive constituents and for the invention of novel allopathic drugs (Adams *et al*., 2009, Bernardini *et al*., 2018, Altemimi *et al*., 2017). Antibiotics and synthetic drugs have key role in treatments of various ailments, but it creates various side effects on human health such as drug resistance, liver and kidney failure (Bernardini *et al*., 2018). The research on traditional uses of medicinal plants species lead to discovery (75 %) of implicit novel herbal drugs discovery and formulation (Calixto, 2005). Plant secondary metabolites such as phenolic and terpenoids compounds possess potential antibacterial, antifungal and antioxidant activities. Secondary metabolites in plant are produced as possible defensive mechanisms against destructive agents (Shabir *et al*., 2011). Most of the commercially available drugs are derived from plants or analogue to natural products. Although, the synthetic antimicrobial drugs considered significant therapeutic agents for inhibiting microbial growth, conversely, misapplication and widespread use of antibiotics result an emerging problem of developing antibiotic resistance against pathogen in human being and also possessed various adverse effects (Shabir *et al*., 2011; Salem *et al*., 2014). So, the medicinal plants are novel source for their basic healthcare to cure various ailments (Hocking, 1962). Medicinal plants provide chances for screening biological activities, and trends in the pharmacological investigations of natural products. Medicinal plants possess variety of secondary metabolites that might be the sources of novelty and prevailing curative agent, which acts as innovative, safer, biocompatible and cost-effective accessible medications (Lindequist *et al*., 2005). In combating new emergence infectious diseases, there is need to search for antibacterial, antifungal, antioxidant and anticancer agents using various methods from medicinal plants (El-Beltagi *et al*., 2018, Al-Tohamy *et al*., 2018).

Researchers from Malaysia reported significant findings that *Ipomoea batatas* has rich content of total phenolic and flavonoids and showed their positive correlation with antioxidant activity (Hue *et al*., 2012). Furthermore, another study reported phenolics attributes in various extract from *Ipomoea batatas* (Indonesia), which exhibited significance antioxidant potential (Fidrianny *et al*., 2018). *Dolomiaea macrocephala* is endemic plant species of alpine vegetation of western Himalayas of Kashmir is locally known as google dhoop (Ahmed *et al*., 2020). *Dolomiaea macrocephala* DC. ex Royle is a medicinal herb belongs to family Asteraceae (also referred to in various publications as *Jurinea dolomiaea* Bioss.) and used in folklore medicine to treat skin bruises, tumor (Lone *et al*., 2015), nervous disorder among the local communities of Kashmir, Pakistan (Ahmed & Akhtar, 2016) and considered as ecological important medicinal plant endemic to Western Himalayas of Kashmir (Ahmed *et al*., 2020). The previous study reported the presence of caffeic acid and apigenin, gallic acid, rutine, catechin, and myricetin in root extract and antioxidant activity (Shah *et al*., 2014). It has been reported that *J. dolomiaea* leaves methanol extract possess antibacterial potential (Dwivedi & Wagay, 2014).

However, there is no information about the total phytochemicals content in various crude extracts from

Dolomiaea macrocephala DC. ex Royle (synonym *Jurinea dolomiaea* Bioss.) leaf and root and their correlation with antimicrobial potential. The present study was designed to investigate phenolics, flavonoids and triterpenoids content in various solvent extracts (petroleum ether, ethyl acetate acetone, methanol, water) from leaves and roots of *D. macrocephala* and their correlation with antimicrobial potential against human pathogenic bacteria and fungi.

Material and Methods

Preparation of plant extracts: The plant specimen of *D. macrocephala* is collected from Himalayas region of District Muzaffarabad, Azad Jammu and Kashmir, Pakistan the specimen was identified (theplantlist, world flora), dried, mounted on herbarium sheet and submitted to AKASH herbarium (AKASH001655) of King Abdullah Campus University of Azad Jammu and Kashmir, Muzaffarabad. The leaves and roots of *D. macrocephala* were air dried in shade for several days and crushed into powder form prior to store in flasks to keep safe them from moisture and light. The pulverized leaves and roots (2 grams/50 mL) were soaked in *Erlenmeyer* flask separately in various solvents such as double distilled water (aqueous), methanol, acetone, ethyl acetate and petroleum ether (all solvents were analytical grade) and were left for three days on orbital shaker under room temperature (25±2). The plant extract was filtered using Whatman No.1 filter paper and filtrate was concentrated by using water bath (45ºC) and stored in refrigerator at 4ºC and subsequent used for phytochemicals content and antimicrobial activities.

Qualitative phytochemical analysis: The preliminary qualitative phytochemical detection for the presence or absence of alkaloids, phenols, flavonoids, terpenoids, cardiac glycosides, tannins, steroids, phytosterol and saponins in the *D. macrocephala* leaves and roots extracts were analyzed using method as described by Harbone (Harbone, 1998, Rattan *et al*., 2016).

Quantitative analysis of phytoconstituents by spectrophotometric method: Preliminary qualitative phytochemical detection indicates presence or absences of major phytochemicals groups and on this basis, the quantitative phytochemical analysis of following major groups were done as follow). The absorbance (optical density) was measured by taking a small aliquot of respective solutions in a quartz cuvette using a double beam spectrophotometer (SPECORD 50 PLUS UV-Vis Spectrophotometer, Germany).

Total phenolic content: The total phenolics were determined followed by the Folin–Ciocalteu method adopted earlier in literature (Velioglu *et al*., 1998). One milliliter of each sample extract mixed with 1 mL of ethanol (95%) and 5 mL of Milli Q water and added 0.5 mL of (50% v/v) Folin–Ciocalteu reagent. After 5 minutes, 1 mL of Na_2CO_3 (5%) was mixed and kept in dark for addition 60 minutes and then the absorbance was read against blank sample (0.5 mL Folin–Ciocalteu reagent and 1 mL of 5% Na_2CO_3) at 725 nm using a spectrophotometer. Quantity of the total phenols contents in unknown samples was calibrated using Gallic acid standard curve and expressed as microgram per gram Gallic acid (GAE) equivalent of the dry weight extract (µg GAE/g DW).

Total flavonoids content: The total flavonoid was determined using aluminium chloride colorimetric method as described in literature, with slight modifications (Chang *et al*., 2002). Each extract samples (1 mL) was separately mixed with mixture 0.5 ml of 5% aluminium chloride, 0.5 ml potassium acetate (0.5 M) and 2.8 ml of double distilled water and left at ambient temperature for 30 minutes. The absorbance was recorded at 415 nm against blank sample (0.5 ml of 5% aluminium chloride, 0.5 ml potassium acetate) using a spectrophotometer. The total flavonoid content in different extracts of *D. macrocephala* was assessed using standard curve of Rutin and stated as microgram equivalents to Rutin per gram dry weight (*µ*g Rut/g DW).

Total triterpenoids: The total triterpenoid content in various solvent extracts of *D. macrocephala* was determined according to described method with slight modifications (Uysal & Aktumsek, 2015). Briefly, a mixture was prepared by adding one mL of sample solution to 0.5 mL vanillin–glacial acetic acid (5%, w/v) and 1 mL perchloric acid and was kept at 60ºC for 10 minutes, followed by cooling in an ice water bath for 15 minutes, afterward 5 mL glacial acetic acids was poured to it and shake well. The absorbance was recorded at 538 nm using a spectrophotometer against blank. The content of total triterpenoids in *D. macrocephala* was presented as Oleanolic acid equivalents (*µ*g OA/g DW) by the standard calibration curve with Oleanolic acid.

Antimicrobial assay

Microorganisms: Six bacterial strains such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Seratia marcesnces* (Gram negative) and *Staphylococcus aureus*, *Streptococcus pyogenes*, *Staphylococcus epidermidis* (Gram positive) were obtained clinically. Two post-harvest fungal pathogens such as *Penicillium expansum* and *Rhizopus stolonifer* were isolated from fruit of *Punica granatum*.

Determination of antibacterial activity: Agar disc diffusion method (Bauer *et al*., 1966) was used to evaluate the antibacterial potential of leaves and roots crude extract. The pure bacterial cultures were renewed by inoculating them on Nutrient agar media by means of streaking method and were incubated at 37°C for 24 hours. To assess the antibacterial potential of plant extracts, about 50 mL of nutrient agar media was decanted in each Petri plates and 100 µL of each bacterial cultural broth were added with the help of micropipette in a respective petri plate and gently mix before allowing to solidifying. The six mm, the paper disc was impregnated in each test sample (1 mg/mL) and placed at delineates position on agar media. Next, plates were incubated at 37°C for 24 hours.

Determination of antifungal activity: Agar disc diffusion method was used to evaluate the antifungal activity with slight modification (Mahboubi & Kazempour, 2015). Briefly, subculture was made from the pure culture of fungal spore in 10 mL sterile distilled water. Cotton was wet by fungal suspension and swabs thoroughly all over the surface on the Potato Dextrose agar medium plates. These plates were then incubated at 27°C for 48 hours prior to antifungal activity. Antifungal assay through disc diffusion method was performed using fungal spore suspension from fresh culture (20 µL) and spread thoroughly all over the surface onto Sabouraud dextrose agar plates before allowing to solidifying. Sterile filter paper disc (6 mm) was dipped in each test sample (1 mg/mL) and placed on respective Petri plates. The plates were incubated at 28 ± 2°C in an incubator for 72 to 96 hours.

Statistical analysis

The experiment was performed in triplicate and zones of inhibition around the disc were measured (millimetres) and mean values plus standard errors of means were presented. Statistical analysis was performed using Origin pro 8 by ANOVA analysis of variance, followed by *post-hoc* Tukey's test at P value < 0.05. The mean value, followed by same letter did not show any significant difference.

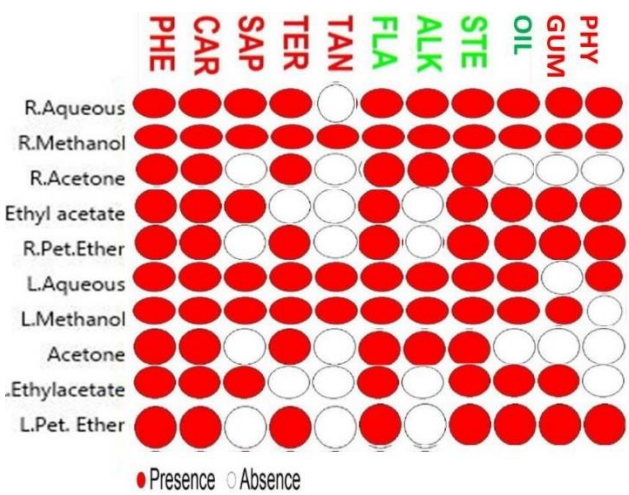

Fig. 1. Dendrogram of qualitative phytochemical analysis of root and leaves of *D. macrocephala.* (L. = leaves / R. = root).
PHY, phytosterol; STE, steroids; ALK, alkaloids; FLA, flavonoids; TAN, tannins; TER, terpenoids; SAP, saponin; CAR, cardiac glycosides; PHE, phenols.

Results

Phytochemical profiling: The preliminary phytochemical screening is the simplest method to detect the important phytochemical groups present in the plant extract. Figure 1 shows the presence or absence of specific phytochemical group in various solvent extract of leaf and root. The qualitative phytochemical analysis is based on visual observation of color changes or precipitation formation after the addition of specific chemical reagent to the solvent extract. The findings revealed that all phytochemical groups were present in methanol and aqueous extract (Fig. 1). The phenolics, flavonoids and triterpenoids were present in all solvent extract of *D. macrocephala*.

Total phenolic content (TPC): The linear regression equation of gallic acid (y=0.6712x-1.2388, R^2=0.996) was used to calculate TPC in various extracts of leaves and roots (Fig. 2). In the present study, the various solvent extract from leaves and roots showed different total phenolics constitute range from 7.12±0.09- 521.28±0.38 µg gallic acid equivalent/g dry weight (Table 1). The maximum phenolic content was found in methanol extract (404.67±0.61 µg GAE/g Dw) followed by aqueous and acetone extract of leaves per gram of dry weight (275.81±0.40 µg and 119.31±0.05 µg GAE/g Dw), respectively. Similarly, total phenolic of roots (521.28±0.38 µg gallic acid equivalent/g dry weight/mL crude extract) was found in methanol followed by acetone (217.17±0.06 µg GAEs /g Dw) and water crude extract (118.66±0.24 µg GAEs /g Dw) from roots.

Total flavonoids content (TFC): The linear regression equation of rutin (y=0.3646x-0.6362, R^2=0.984) was used to calculate TFC in various extracts of leaves and roots (Fig. 2). The total flavonoids content in various solvent crude extract from leaves and roots exhibited range from 0.31±0.02-489.29±0.24 µg rutin equivalent/g dry weight of sample extract (Table 2). The results showed that maximum flavonoid was found in methanol and acetone (489.29±0.24 and 449.93±0.10 µg rutin equivalent/g Dw) crude extract of roots followed by methanol crude extract of leaves. Moreover, maximum total flavonoid was recorded in aqueous crude extract of roots (48.03±0.11 µg rutin equivalent/g Dw) as compared to aqueous crude extract of leaves (30.01±0.25 µg rutin equivalent/g Dw). Total flavonoids content was accounted in least quantity in ethyl acetate crude extract of roots (0.31±0.02 µg rutin /g Dw) and petroleum ether crude extract of leaves (0.73±0.03 µg rutin /g Dw).

Total triterpenoids content (TTC): The linear regression equation of oleanic acid (y=0.0437x-0.0055, R^2 = 0.9496) was used to estimate total triterpenoids content in various extracts of leaves and roots (Fig. 2). The total triterpenoids content in various solvent crude extract from leaves and roots were recorded in the range of 3.32±0.06-30.52±0.64 µg rutin equivalent/g dry weight of sample extract (Table 2). Highest triterpenoids were recorded in petroleum ether extract (30.52±0.64) of root (30.52±0.64) and leaves (20.53±0.03) followed by methanol and ethyl acetate extract of leaves. On the other hand, aqueous and acetone extract of both leaves and roots showed least quantity of triterpenoids content.

Antibacterial activity: Figure 3 displays that the maximum zone of inhibition was recorded by Petroleum ether extract of leaves (13.5±0.9) followed by aqueous (12.33±0.8) and methanol extract (11.83±0.7) against *S. aureus*. In addition, the methanol extract exhibited maximum inhibition zone against *S. pyogenes* (12.5±0.4) and *P. aeruginosa* (11.93±0.9). The zone of inhibition (10.83±0.4) against *S. epidermidis* was recorded in case of leaves aqueous extract.

Table 1. Total bioactive compounds in different solvent extracts obtained from leaves of *D. macrocephala*.

Solvents	Total phenolics (µg GAE/g extract)	Total flavonoids (µg Rut/g extract)	Total triterpenoids (µg OAE/g extract)
Aqueous	275.81 ± 0.40[a]	30.01 ± 0.25[a]	4.09 ± 0.33[a]
Methanol	404.67 ± 0.61[b]	363.59 ± 0.36[b]	8.68 ± 0.13[b]
Acetone	119.31 ± 0.05[c]	114.73 ± 0.51[c]	3.32± 0.06[c]
Ethyl acetate	07.12 ± 0.09[d]	01.05 ± 0.01[d]	8.76 ± 0.02[b]
Petroleum ether	64.38 ± 0.02[d]	00.73 ± 0.03[d]	20.53 ± 0.03[d]

Results expressed are means ± standard error of means (SEM) from three independent experiments
Note: Any two means with the same letter in a column are non-significantly different at $p<0.05$ (Tukey's test)

Table 2. Total bioactive compounds in different solvent extracts obtained from roots of *D. macrocephala*.

Solvents	Total phenolics (µg GAE/g extract)	Total flavonoids (µg Rut/g extract)	Total triterpenoids (µg OAE/g extract)
Aqueous	118.66 ± 0.24[a]	48.03 ± 0.11[a]	5.21 ±0.01[a]
Methanol	521.28 ± 0.38[b]	489.29 ± 0.24[b]	6.58 ± 0.1[a]
Acetone	217.17 ± 0.06[c]	449.93 ± 0.10[c]	3.85± 0.02[b]
Ethyl acetate	33.19 ± 0.07[d]	00.31 ± 0.02[d]	7.18± 0.03[a]
Petroleum ether	71.19 ± 0.04[e]	02.14 ± 0.08[d]	30.52± 0.64[c]

Results expressed are means ± standard error of means (SEM) from three independent experiments
Note: Any two means with the same letter in a column are non-significantly different at p<0.05 (Tukey's test)

The aqueous roots extract showed potential zone of inhibition against *Pseudomonas aeruginosa* (13.83±0.4) followed by *S. epidermidis* (12.33±0.2) and *S. aureus* (12±0.3). The methanol extracts showed good antibacterial activities against *S. aureus* (11.83±0.6) followed by *P. aeruginosa* (11.93±0.64). The minimum inhibition zone was recorded against *K. pneumonia* (9.6±0.2) (Fig. 4). The other crude extract showed least antibacterial activities against all tested bacteria.

Antifungal activity: The antifungal activities of aqueous extract of leaves and roots were tested against *Rhizopus stolonifer* and *Penicillium expansum*. The results revealed the growth of *P. expansum* is potentially inhibited by *D. macrocephala* aqueous leaves (22±0.66 mm) and roots crude extract (28±0.33 mm) (Fig. 5). The methanol extracts from leaves and roots showed least inhibitory effect against both fungal strains. However, other solvents extract were found inactive against tested fungal strains.

Correlation between total phytochemicals content and antimicrobial activities: The Pearson's correlation analysis method was adopted to assess the strength and direction between total phenolic and flavonoid content in leaves and roots extracts with their antimicrobial activities variables. Figure 6 shows that the statistical analysis revealed significant positive correlation between total phenolics (0.348, (R2 = 0.121) and flavonoids (0.169, (R2 = 0.029) contents from leaves and their antibacterial activities at significant level *P* 0.001. Similarly, statistically significant positive correlation between total phenolics (0.235, (R2 = 0.055) and negative correlation with total flavonoids (-0.022, R2 = 0.00) contents from roots and their attributes with antibacterial activities were recorded. It was also recorded negative correlation triterpenoids content in roots (-0.115, R2 = 0.024) and leaves (-0.010, R2 = 0.00) showed with their antibacterial activities. Moreover, the attributes of total phenolics and triterpenoids in leaves and roots of showed negative correlation with antifungal activities.

Discussion

The presence of these important secondary metabolites attributed for antibacterial and antifungal activity (Hussain *et al*., 2011). The preliminary detection of similar phytochemicals was also reported in other studies (Banothu *et al*., 2017; Nayak *et al*., 2017). Plant phenolics and triterpenoid saponins constitute the important secondary metabolites, which possess antioxidant, anticancer and antimicrobial properties (Kabera *et al*., 2014, Ferrazzano *et al*., 2011). Therefore, it was reasonable to investigate their profile in various solvent extract from leaves and roots of *D. macrocephala*. The present findings confirmed the higher contents of phenolics in methanol, aqueous and acetone crude extracts from both leaves and roots as compared to less polar solvents. While the flavonoids content were recorded highest in methanol and acetone crude extracts of roots as compared to aqueous and less polar solvents. In contrary to this study, previous study reported that chloroform and ethyl acetate fractions from roots possessed highest flavonoids and phenolics content, but similarly results reported in a previous study that less polar solvent (n-hexane) yield lowest quantities of these compounds (Shah *et al*., 2014).

The antibacterial activity of leaves and roots solvents extracts was evaluated against "Gram-positive" and "Gram-negative" bacteria. Form the present finding, it was perceived that Gram-positive bacteria were most susceptible than Gram-negative. The present results showed good agreement with previous findings (Somarathna *et al*., 2018). The present study revealed that the aqueous and petroleum ether crude extract of leaves exhibited good antibacterial potential against *S. aureus*. However, lowest flavonoids content and lower phenolic content was obtained in petroleum ether crude extract of leaves.

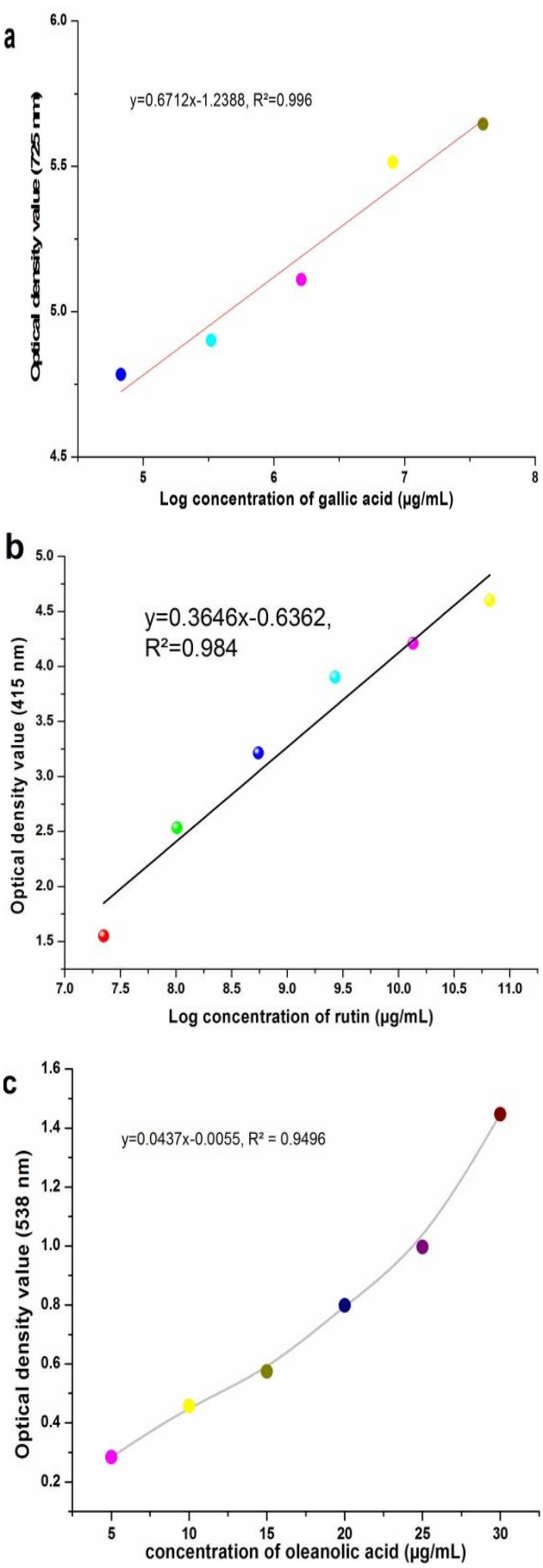

Fig. 2. Calibration curve of standard (a) gallic acid, (b) rutin, (c) oleanolic acid.

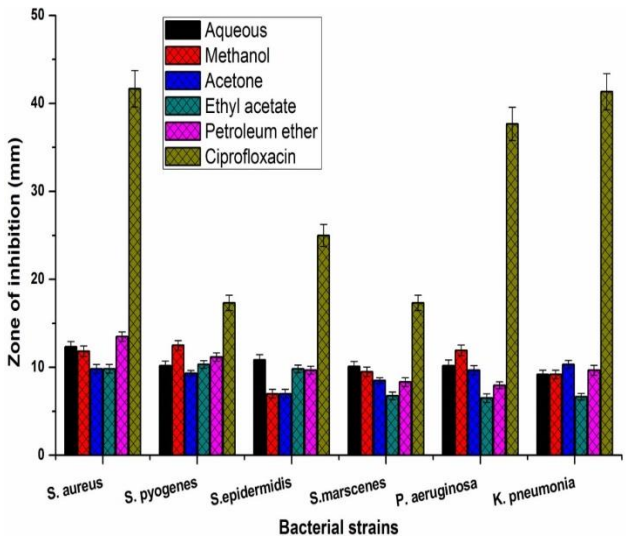

Fig. 3. Antibacterial activity of various solvent extracts from *D. macrocephala* (leaves).

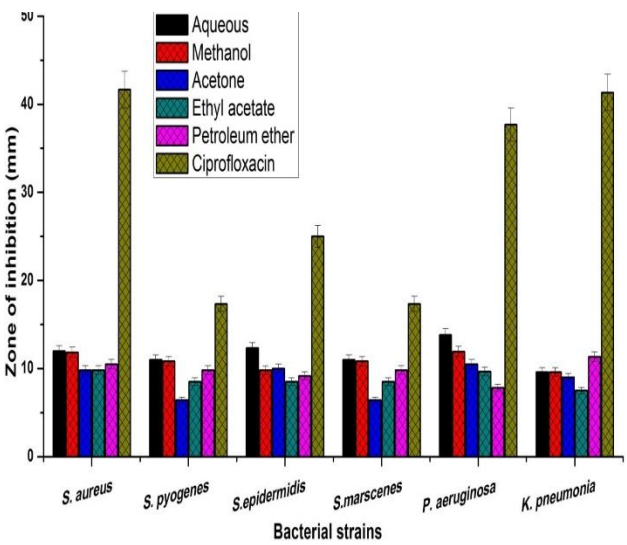

Fig. 4. Antibacterial activity of various solvent extracts from *D. macrocephala* (roots).

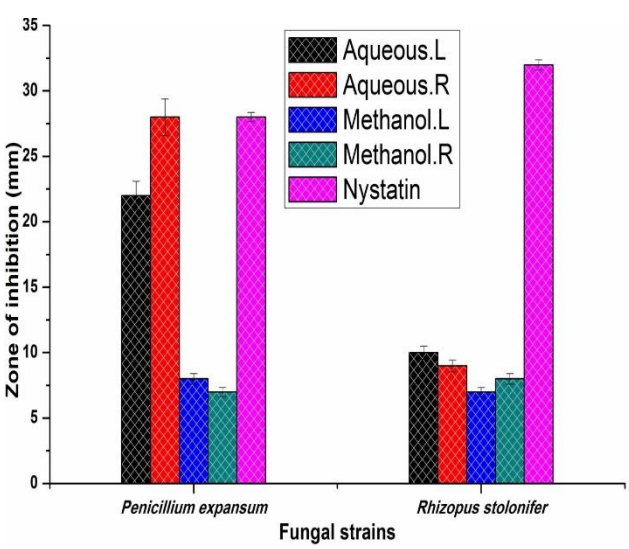

Fig. 5. Antifungal activity of solvent extracts from leaves (L) and roots (R) of *D. macrocephala*.

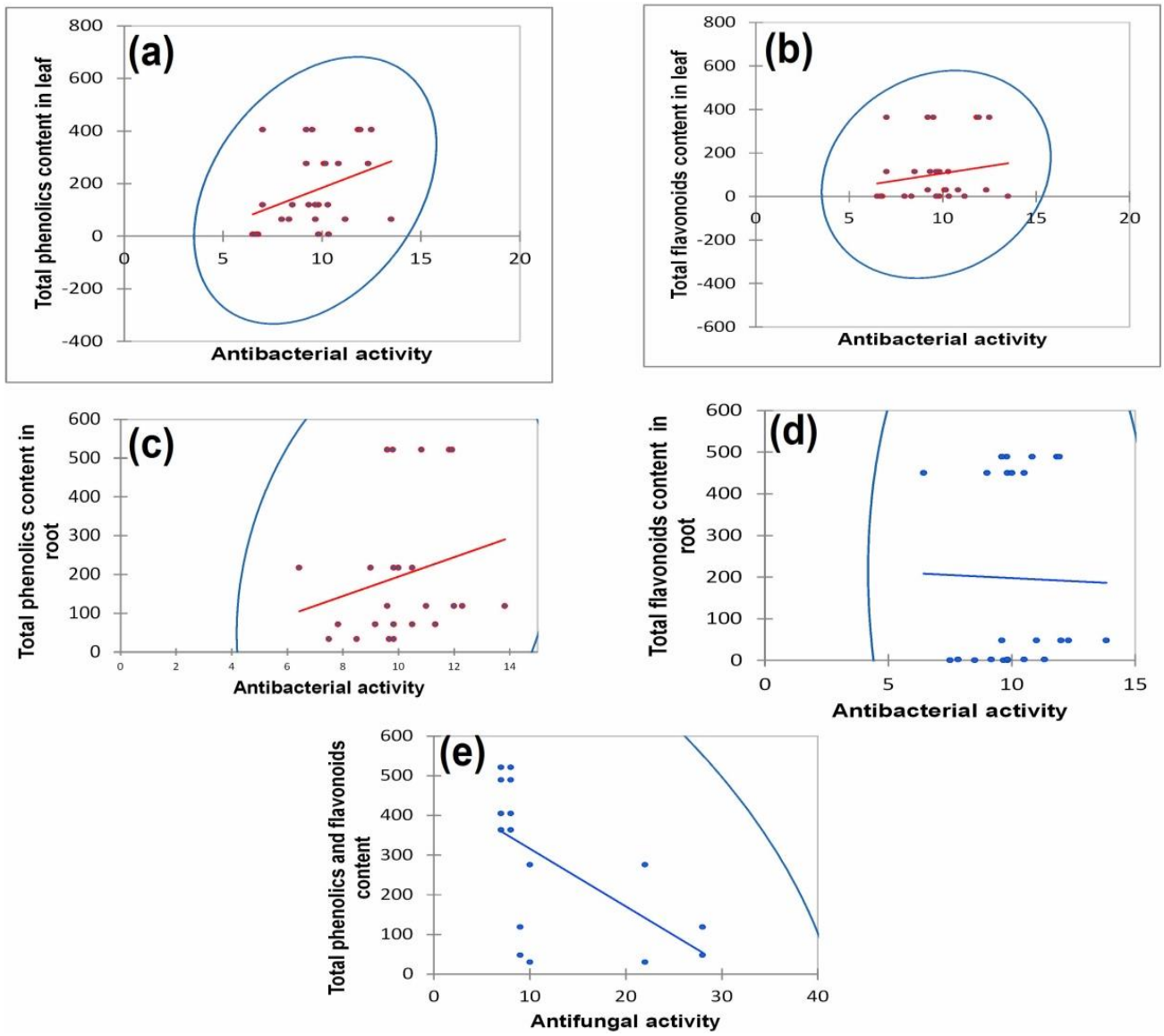

Fig. 6. Scatter plot showing the Pearson's correlation between total phenolics and flavonoids content in leaves (a, b), and roots (c, d) with antibacterial and antifungal activity (e).

In addition, the present finding exhibit that the aqueous crude extracts from root showed remarkable zone of inhibition against "Gram-positive" *S. aureus* and "Gram-negative" *P. aeruginosa*. Similarly, aqueous root extract also showed pronounce growth inhibition against all tested bacteria except *K. pneumonia*. The remarkable antibacterial activity of root aqueous extract was due to presence of higher phenolic content. Moreover, methanol crude extract of leaves and roots exhibited good antibacterial growth inhibitory effect against all tested bacterial strains except *S. epidermidis* and *K. pneumonia* and *S. marcesnces*. The good antibacterial potential of methanol crude extracts from leaves and roots was due to highest content of phenolic and flavonoids. The present findings also supported by statistical analysis of correlation between phenolics content and antibacterial activity. The total phenolics and flavonoids content in (leaves and roots) various crude extracts showed significant positive correlation with antibacterial activity. However, flavonoids content showed negative correlation with antibacterial activity. The present study is good accordance with the previous reported studies on phenolics content and their biological activities (Shabir *et al*., 2011, Fidrianny *et al*., 2018). Various researchers reported in previous studies that aqueous and methanol crude extract of plant contain higher phenolics and flavonoids content as compared to non-polar solvent (Shabir *et al*., 2011, Shah *et al*., 2014, Altemimi *et al*., 2017). Phenolics and flavonoids are chiefly reported in plants and have beneficial effect on human health (Bernardini *et al*., 2018). Various studies on phenolics and flavonoids and their derivatives have shown a wide range of antibacterial, antifungal, anti-inflammatory, anti-allergic, anti-oxidants and antiviral activities (Shabir *et al*., 2011, Velioglu *et al*., 1998, Somarathna *et al*., 2018). Similar study reported that these bacteria showed sensitiveness to various crude extract of *Allium sativum* and *Zingiber officinale* tested against them (Awan *et al*., 2017). The finding of present results revealed that various crude extracts of *D. macrocephala* showed good results as compared to previously reported study of medicinal plants

against same test bacterial strains (Awan *et al*., 2013). From the present findings it was revealed that only aqueous and methanol extract of *D. macrocephala* (leaves and roots) exposed remarkable significant antifungal activity against *Rhizopus stolonifer* and *Penicillium expansum*. Roots and leaves aqueous extract from *D. macrocephala* exhibited excellent zone of inhibition against *P. expansum*. However, other solvents extractions were found inactive in restricting the fungal growth of post-harvest filamentous fungi. Previous studies reported that antifungal activities of plant extracts were due to secondary metabolites that is alkaloids, phenolic and flavonoids (Noshad *et al*., 2018). Pearson's correlation analysis revealed that total phenolics, flavonoids and triterpenoids content in aqueous and methanol extracts from leaves and roots showed negative correlation. The previous studies on phytochemical analysis of *Jurinea* species reported that saponins and sesquiterpenes are rich in this genus (Kumar & Agnihotri, 2018, Taherkhani & Rustaiyan, 2016). Moreover, some author investigated that saponins and terpenoids from medicinal plants showed inhibitory effect on fungal growth (Noshad *et al*., 2018, Rattan *et al*., 2016). Overall, the statistical analysis of correlation between total phenolics content in various crude extracts and antibacterial activity showed a linear relationship (Fig. 6). The coefficient of determination value of total phenolics (R2 = 0.121), flavonoids (R2 = 0.029) content in leaves and total phenolics (R2 = 0.055) content in root showed 12%, 2.9% and 5.5% of the difference in their phenolics (various solvent extracts) content respectively with respect to their antibacterial activity. It should be noticed that such studies have significant contributed to the phenolics of medicinal plants with their biological activities (Shabir *et al*., 2011, Russo *et al*., 2015).

Conclusions

The present findings revealed that various solvents extracts possessed significant amounts of phenolic and flavonoids content in both leaves and roots of *D. macrocephala*. The various solvent extracts from leaves and roots were found potent antibacterial agents against human pathogenic bacteria. Moreover, aqueous and methanol extract of leaves and roots also exhibited good activities against fungus *Rhizopus stolonifer* and *Penicillium expansum*. The statistical analysis with regard to antimicrobial activities and phytochemical content were also validated with the present findings. Thus, further research should be conducted to determine the efficacy of various solvent extracts against other pathogenic bacteria and fungal strains. In addition, it is suggested that further study should be conducted using technique such as HPLC, GC or GC-MS analysis for molecular fingerprinting of bioactive compounds in order for novel drug development and drug discovery.

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