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Phytochemical Screening and Antimicrobial Evaluation of Various Fractionates of Ethanolic Extract of *Launaea sarmentosa* (Willd.), in the Search of Possible Candidates to Multidrug Resistant Microbes

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Abstract:

Presently, misuse of antibiotics causes the development of microbial resistance to many drugs and consequently increased number of multidrug resistant (MDR) microbes has become a global disaster in human health. For reversing the MDR characteristics, plants containing diverse bioactive compounds play a crucial role. This study was carried out to evaluate the phytochemicals and antimicrobial activities of pet ether, ethyl acetate and chloroform fractionates (PEF, EAF & CHF) of crude ethanol extract of the plant *Launaea sarmentosa* (Willd.). Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, steroids, saponins, tannins in CHF in large extent. Disc Diffusion assay was performed to evaluate antimicrobial activity of fractionates against eight-gram (+ve) and ten gram (-ve) bacteria and six fungi. The CHF of the crude extract exhibited highest activity to gram (-ve) bacteria confirmed by measuring the diameter of the zone of inhibition; *Shigella boydii* (25.80 ± 0.53mm), *Shigella shiga* (25.42 ± 0.04mm), *Salmonella typhi* (25.20 ± 0.04mm), *Escherichia coli* (24.53 ± 0.02mm) etc. and appreciable activity to the gram (+ve) MDR strain; *Klebsiella pneumoniae* (23.12 ± 0.03mm), *Staphylococcus aureus* (23.12 ± 0.01mm) etc. Among all fractionates, the CHF was found to have the lowest (32µg/ml) Minimum Inhibitory Concentration (MIC) value (determined by Serial Tube Dilution technique). Each of the fractionates was also found to demonstrate good antifungal activity with 10.68 ± 0.33 to 16.57 ± 0.07mm inhibition zones at 500µgm/disc. The presence of a large number of phytoconstituents and remarkable antimicrobial activities have made the plant *Launaea sarmentosa* (Willd.), a promising candidate for the development of new and effective antimicrobial agent.

Keywords: Antibacterial, Antifungal activity, Disc Diffusion method, *Launaea sarmentosa* (Willd.), MIC, Phytochemical screening.

INTRODUCTION

The inappropriate use of antibiotics in the treatment of infectious diseases has led to the emergence of antibiotic resistant pathogens worldwide. Antibiotic resistance has become a serious and widespread problem that causes high morbidity, mortality and health care cost in every year [1-5]. This phenomenon results in reduced effectiveness of the treatment regimens, delayed illness of patient and even it leads to the complete failure of treatment [6]. The situation is much more worsening in underdeveloped and developing countries day by day due to lack of proper knowledge of peoples, registered physicians or consultants [7-9]. Although, new classes of antibiotics are available in the market it is still somewhat difficult to reduce the antibiotic resistance using the synthetic drugs. In this respect, nature serves as a repository of remedial agents from the very ancient time and medical science largely depends on the drugs that are originated from the natural resources [10]. Plants have played a crucial role against many diseases in human [11, 12]. A number of reports show that the crude extracts of different parts of medicinal plants possess good antimicrobial properties. Therapeutic activity of these plants is largely due to various bioactive substances that produce a distinct physiological effect on the human body [13, 14]. Medicinal plants contain a large number of bioactive compounds like alkaloids, flavonoids, phenolics, terpenoids, coumarin, tannins, essential oils, lectins, sterols, polypeptides, and polyacetylenes [15-18]. These biomolecules are used as a starting material of antibiotics synthesis for the treatment of infectious diseases [19].

Launaea sarmentosa (Willd.), synonym *L. pinnatifida* (Cass.), belonging to the Asteraceae family, is locally known as kulhafila in the Maldives. It is a creeping, perennial procumbent herb, native to tropical Indian coastlines [20, 21]. Leaves of *L. sarmentosa* (Willd.) with simple leaflets (12-15 cm long) are slightly bitter in taste and have characteristic odor. Traditionally, it has gained extensive popularity in the treatment of abdominal disorders, urinary infections [20]. The whole plant is used in rheumatoid arthritis, gout and the leaf in rheumatism and skin related disorders [22]. It is also used as nutritious vegetable or supplement of mother's milk after child birth [23]. Previous phytochemical studies of *L. sarmentosa* (Willd.) have confirmed the presence of alkaloids, carbohydrates, amino acids, steroids, glycosides, flavonoids, tannin, etc. It is reported that such compounds exhibit anti-inflammatory, antioxidant, and hepatoprotective activities [20, 24, 25]. However, there are limited detailed of this plant for their potential role as antimicrobials and phytochemical entities as therapeutic agents for pathogenic bacteria and fungi. It demands a thorough investigation of *L. sarmentosa* (Willd.) for its antimicrobial potentiality as well as phytochemicals and secondary metabolite contents. Keeping this in mind, the

present study was aimed to investigate the presence of phytochemicals as well as the antibacterial and antifungal activities of various fractionates (PEF, EAF & CHF) of *L. sarmentosa* (Willd.) crude ethanol extract.

MATERIAL AND METHODS

Plant collection

The herb, *Launaea sarmentosa* (Willd.), was collected from Kurigram district of Bangladesh in the month of December, 2019. The collected herb was handled with standard storage protocols and transported with plastic sheet wrapping. Authentification of the plant sample was done by an expert taxonomist and a voucher specimen was deposited for preservation in the National Herbarium, Mirpur, Dhaka-1216, Bangladesh, with the accession number DACB: 38312.

Plant material extraction and fractionation

After collection, the whole plant, *Launaea sarmentosa* (Willd.), was washed thoroughly with distilled water, air dried at room temperature (23-27^{o}C) under shade, oven dried at 40^{o}C and pulverized with a grinding machine. The ground material was packed in a plastic bag and kept in cool and dark place until extraction. The powdered plant (700 gm) was submerged in 2.5 litres ethanol in a suitable flat bottomed flask at room temperature and subjected to occasional shaking and stirring for five days [26]. The extract was separated from the marc using fresh cotton plug and then filtered through Whatman No. 1 filter paper (Whatman Ltd., England). The above maceration procedure was repeated three times to comprehensively extract the plant material.

For complete removal of solvent, the filtrates were then evaporated to dryness in vacuum by a rotary evaporator at 40-50^{o}C. Using a lyophilizer (Labfreez, China) the extract was further concentrated to dryness by freeze-drying. Using separating funnel the prepared mass was then fractionated by solvent-solvent partitioning with petroleum ether (PE), ethyl acetate (EA) and chloroform (CH) [27-30]. Each fraction were then redried under reduced pressure in rotary evaporator, kept into respective glass vials and stored in a refrigerator at -4^{o}C until use for investigation.

Growth media and conditions

Nutrient agar media (Difco Laboratories) P^{H} 7.2, Nutrient broth media (Difco Laboratories) P^{H} 6.2 and Sabouraud dextrose agar media (Biolife Vole Monza) P^{H} 5.6 were used for antibacterial screening, MIC determination and antifungal activity assay respectively [31, 32].

Test microorganisms

The antibacterial activities of the plant extracts were evaluated against eight gram (+ve) bacteria namely *Staphylococcus aureus*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, and *Clostridium perfringens* and ten gram (-ve) bacteria namely *Escherichia coli*, *Pseudomonus aeruginosa*, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei*, *Shigella boydii*, *Shigella shiga*, *Salmonella typhi*, *Salmonella paratyphi* and *Vibrio mimicus*. The antifungal activity was tested against six fungi namely *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus ustus*, *Candida albicans*, *Rizopus oryzae* and *Trichophyton rubrum*. All of these microorganisms were collected from the Microbiology Research Laboratory of Pharmacy Department, University of Rajshahi, Rajshahi-6205, Bangladesh.

Standard antibiotic discs

Kanamycin (30µg/disc, Hi-media, India), and Ampicillin (30µg/disc, Hi-media, India), were used in this study as standards to compare the antibacterial activity of the extracts. Besides, the modern antifungal drug, Ketoconazole (20µg/disc, Hi-media, India), and Griseofulvin (20µg/disc, Hi-media, India) were included to compare the antifungal activity. All of these antibiotic discs were purchased commercially.

Phytochemical investigation

The petroleum ether, chloroform and ethyl acetate fractionates (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.) crude ethanolic extract, were subjected to qualitative phytochemical screening for the presence or absence of different phytoconstituents or secondary metabolites (Table 1). In each case, the test solution was prepared at 10% (w/v) concentration in distilled water unless otherwise stated in individual test. This investigation was carried out according to the standard protocols [33-37].

Tests for alkaloids (Dragendorff's test): Around 150mg of three different extracts (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.), were boiled in few ml methanol and filtered. Then, 1% HCl was added followed by the addition of few drops of Dragendorff's reagent (Potassium bismuth iodide solution). Reddish brown precipitate indicated the evidence of presence of alkaloids. **Mayer's test:** About 3 ml of plant extracts were kept in water bath for 20 min. After cooling and filtering the solution, addition of few drops of Mayer's reagent (Potassium mercuric iodide solution) showed the appearance of cream colored precipitate that revealed the presence of alkaloids. **Wagner's test:** Addition of Iodine solution in Potassium Iodide (Wagner's reagent) to the extracts produced a reddish brown precipitate that showed the presence of alkaloids.

Test for glycosides (Keller Killiani test): About 2 ml of chloroform was added to 3 ml of each fractionates of the extract and mixed. Then 1 ml of concentrated sulfuric acid was added very carefully along the side of the test tube to form a lower layer. Appearance of a reddish brown color at the interface indicated the presence of glycosides.

Tests for flavonoids (Alkaline reagent test): To each of the test solutions (approximately 3 ml), prepared from each fractionates of the crude extracts, 1ml of 10% sodium hydroxide solution was added, appearance of an intense yellow colour which became colourless upon addition of few drops of dilute acetic acid confirmed the test. **Ferric chloride test:** To each of the test solutions, few drops of ferric chloride solution were added. An intense green coloration indicated the presence of flavonoids.

Tests for steroids and triterpenoids (Salkowski's test): Each crude fractionates (PEF, EAF & CHF) were treated in chloroform with few drops of concentrated sulphuric

acid, shaken well and allowed to stand for 10 minutes. Formation of red and yellow colour in the lower layer indicated the presence of steroids, and triterpenoids respectively. **Libermann-Burchard's test:** To 1ml of each fractionates; 2ml of acetic anhydride was added, boiled and cooled. 2ml concentrated sulphuric acid was added very slowly along the side of the test tube. Appearance of a brown ring at the interface of the two layers and a blue green upper layer indicated the presence of steroids. Additionally, the formation of a deep red colour indicated the presence of triterpenoids.

Tests for phenolic compounds (Ferric chloride test): To 3ml of each crude extract, few drops of neutral 5% ferric chloride solution were added. A dark bluish green precipitate indicated the presence of phenolic compounds.

Lead acetate test: Appearance of white precipitate after addition of few drops of 10% lead acetate solution to each of the test samples indicated the presence of phenolic compounds.

Tests for Carbohydrates (Molisch's test): A general test for carbohydrates is the Molisch's test which is given by almost all of the carbohydrates. Few drops of alcoholic α-napthol were added to 1ml of test sample prepared from the crude extracts. After this, concentrated sulfuric acid (0.3 ml) was added very slowly along the side of the test tube. Appearance of purple to violet colored ring indicated the presence of carbohydrates.

Test for saponins (Frothing test): 5ml of distilled water was added to 1ml of PEF, EAF and CHF separately and the resulting mixture was shaken vigorously until it froths. Samples showing froth were warmed. Persistent foam formation indicated the positive results for saponins.

Test for tannins (Braymer's test): About 3mg of PEF, EAF and CHF taken separately in a test tube was boiled in 5ml of water and then filtered. 2 to 3 drops of 0.1% ferric chloride was added and formation of brownish green or a blue-black precipitate indicated the presence of tannins. **Gelatin test:** In 1ml of test solution, five drops of 1% gelatin containing 10% sodium chloride was added. Formation of white precipitates indicated the evidence of tannins.

Antibacterial susceptibility assay

The antibacterial activity of each crude extracts (PEF, EAF & CHF) was carried out against eight pathogenic gram (+ve) and ten gram (-ve) bacteria by the agar Disc Diffusion method (Table 2) [29, 38]. Discs (6 mm diameter, filter paper) impregnated with the test materials prepared from each PEF, EAF and CHF at 500µg/ml concentration were placed on the nutrient agar medium in sterile petridishes which were uniformly seeded with the test microorganisms under aseptic condition. To compare, as well as to ensure the specificity of the results, standard antibiotic discs (Kanamycin (30µg/disc), Ampicillin (30µg/disc), and blank discs (impregnated with 10µl solvents followed by drying off) were used as a positive and negative control respectively. The petridishes were kept in a refrigerator at 4ºC for 18 hours to allow maximum diffusion of test material into the surrounding media. After inoculation, petridishes were incubated overnight at 37ºC for proper growth of bacteria. The activity was determined by measuring the diameter of the zone of inhibition expressed in millimeter (mm), the greater the diameter, the more antibacterial potential of the fractionate.

Determination of Minimum Inhibitory Concentration (MIC)

The Serial Tube Dilution technique [39, 40] was used to determine MIC value of PEF, EAF and CHF of *Launaea sarmentosa* (Willd.) crude ethanolic extract against all the pathogenic bacteria. The potentiality of antibacterial activity was investigated by measuring the MIC value in µg/ml. An extract with a lesser MIC value, is the more potent antibacterial. Each fractionates of the extract was dissolved in 2 ml distilled water to obtain stock solution of 512µg/ml concentration. 1ml of this prepared stock solution was transferred to test tube containing 1ml nutrient broth medium to have 256µg/ml concentration in the 1st test tube and mixed well. This process of serial dilution was carried on up to 9th test tube to have a concentration of 128µg/ml, 64µg/ml, 32µg/ml, 16µg/ml, 8µg/ml, 4µg/ml, 2µg/ml, 1µg/ml consecutively. Then, 1 drop suspension of test organisms (0.02 ml) was added to each of the 9 test tubes, contaminating the test sample and nutrient broth medium. Concurrently, 9 test tubes were prepared as positive control or standards for each of Kanamycin, and Ampicillin at the same concentration as that of sample. Distilled water with 3 drops of Tween 80 was used as negative control in each case. Sterility was maintained very strictly throughout the procedure. After 18 hours incubation at 37ºC, the test tubes were then observed for bacterial growth. Growth was observed in those test tubes where the concentration of the extract or the antibiotics were below the inhibitory level (Table 3) of the test bacteria and the broth of those test tubes were observed turbid due to growth of bacteria.

Antifungal screening

The Disc Diffusion method [29, 38] adopted for the determination of antifungal activity of PEF, EAF and CHF against fungi was same as that of the bacteria. Antifungal investigation was performed against six pathogenic fungi at a concentration of 500µg/disc as described in antibacterial screening section. Instead of nutrient agar medium, Sabouraud dextrose agar was used. Standard disc of antifungal agents like Ketoconazole (20µg/disc), and Griseofulvin (20µg/disc) and blank discs (impregnated with solvents used for the preparation of test sample of the fractionates) were used as a positive and negative control respectively (Table 4).

RESULTS

Phytochemical analysis

In phytochemical evaluation, the whole plant *Launaea sarmentosa* (Willd.), were tested qualitatively for their phytoconstituents. It was found that the plant contains almost all the biologically important secondary metabolites like alkaloids, glycosides, flavonoids, steroids, triterpenoids, phenolic compounds, carbohydrates, saponins and tannins (Table 1). Different

fractionates (PEF, EAF & CHF) obtained from the crude ethanol extract were found to contain these phytochemicals to a different degree according to the nature of the solvents and the chemical group of the compounds. This quantitative assumption was based on the intensity of the colored reaction product of the test sample compared to control in each case. In case of PEF, secondary metabolites were present in minor extent and alkaloids, flavonoids, phenolic compounds and tannins were not detected (Table 1). Biomolecules except glycosides, saponins and tannins were detected in a moderate amount in the ethyl acetate fractionate (EAF). Chloroform fraction (CHF) was recorded to be the highest and found to contain almost all the phytoconstituents to a moderate and significant extent.

Antibacterial susceptibility

Various fractionates (PEF, EAF & CHF) at 500µg/disc concentration of *Launaea sarmentosa* (Willd.) crude ethanol extract, were observed to have appreciable antibacterial activities against eight pathogenic gram (+ve) and ten gram (-ve) bacteria as was confirmed by the traditional disc diffusion assay (Table 2). Among the three fractionates, the CHF exhibited greater activity to gram (-ve) bacteria. The highest inhibition zone was found to be 25.80 ± 0.53mm in diameter against *Shigella boydii* followed by *Shigella shiga, Salmonella typhi, Escherichia coli, Salmonella paratyphi, Pseudomonus aeruginosa*, *Shigella flexneri*, *Shigella dysenteriae*, *Shigella sonnei,* and *Vibrio mimicus* with the inhibition zones of 25.42 ± 0.04mm, 25.20 ± 0.04mm, 24.53 ± 0.02mm, 24.32 ± 0.05mm, 24.25 ± 0.01mm, 24.23 ± 0.03mm, 24.22 ± 0.01mm, 23.24 ± 0.01 mm and 23.17 ± 0.07mm respectively. In addition, the CHF had moderate activity to gram (+ve) MDR strains like *Klebsiella pneumoniae* with 23.12 ± 0.03mm, and *Staphylococcus aureus* with 23.12 ± 0.01mm zone of inhibition. Table 2 showed that among the eight gram (+ve) bacteria tested, *Bacillus cereus* showed lowest sensitivity to the PEF with the inhibition zone of 15.80 ± 0.01mm diameter.

Minimum Inhibitory Concentration (MIC)

The MIC values of PEF, EAF and CHF of *Launaea sarmentosa* (Willd.) ethanol extract, were found to inhibit the growth of the tested microorganisms at 128 to 256µg/ml, 64 to 128µg/ml and 32 to 64µg/ml respectively (Table 3). The lowest MIC (32µg/ml) was observed in the case of CHF against gram (-ve) strains; *Escherichia coli, Shigella boydii, Shigella shiga, Salmonella typhi, Salmonella paratyphi.*

Antifungal screening

This study revealed that PEF, EAF and CHF of the *Launaea sarmentosa* (Willd.) crude ethanol extract demonstrated significant antifungal activities against human pathogenic fungi (Table 4). Maximum inhibition was obtained by the CHF against *Aspergillus niger* followed by *Rizopus oryzae*, *Candida albicans* and *Aspergillus ustus* with the inhibition zone of 16.57 ± 0.07mm, 16.08 ± 0.03mm, 15.92 ± 0.02mm and 15.34 ± 0.01mm in diameter respectively. The EAF was found to be moderately effective against the tested fungi. Besides, the PEF had less antifungal activities compared to others with the inhibition zone less than 13mm.

Table 1: Phytochemical analysis of three fractionates (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.) crude ethanol extract.

Phytoconstituents (secondary metabolites)	Chemical tests performed	Pet. Ether fractionates (PEF)	Ethyl Acetate fractionates (EAF)	Chloroform fractionates (CHF)
Alkaloids	Dragendorff's test	–	++	+++
	Mayer's test	–	++	+
	Wagner's test	–	++	+++
Glycosides	Keller-Killiani test	+	–	++
Flavonoids	Alkaline reagent test	–	++	++
	Ferric chloride test	–	++	++
Steroids	Salkowski's test	+	+	+
	Liebermann Burchard's test	+	+	++
Triterpenoids	Salkowski's test	+	+	+++
	Liebermann Burchard's test	+	+	++
Phenolic compounds	Ferric chloride test	–	++	+++
	Lead acetate test	–	++	++
Carbohydrates	Molisch's test	+	++	++
Saponins	Frothing test	+	–	+
Tannins	Braymer's test	–	–	+
	Gelatin test	–	–	++

'–' (not present), '+' (present to a minor extent), '++' (present at a moderate extent), '+++' (present at a significant extent)

Table 2: Antibacterial activities of three fractionate (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.) crude ethanol extract.

Bacterial strains tested	Inhibition zone in diameter (mm) of three fractionates (500µg/disc)			Ampicillin (30µg/disc)	Kanamycin (30µg/disc)
	PEF	EAF	CHF		
Gram (+ve)					
Staphylococcus aureus	16.20 ± 0.03	19.25 ± 0.02	23.12 ± 0.01	30.21 ± 0.10	35.24 ± 0.34
Streptococcus agalactiae	17.30 ± 0.04	20.30 ± 0.03	22.62 ± 0.07	32.47 ± 0.01	33.93 ± 0.05
Klebsiella pneumoniae	15.90 ± 0.23	19.28 ± 0.01	23.12 ± 0.03	31.33 ± 0.01	34.95 ± 0.02
Streptococcus pyogenes	15.85 ± 0.13	19.60 ± 0.03	22.42 ± 0.02	32.51 ± 0.04	35.61 ± 0.01
Bacillus cereus	15.80 ± 0.01	19.29 ± 0.07	23.11 ± 0.04	30.25 ± 0.03	34.86 ± 0.03
Bacillus megaterium	16.34 ± 0.33	20.25 ± 0.06	23.08 ± 0.01	32.41 ± 0.33	34.93 ± 0.35
Bacillus subtilis	16.25 ± 0.05	19.58 ± 0.05	23.13 ± 0.05	31.72 ± 0.43	35.41 ± 0.03
Clostridium perfringens	16.00 ± 0.03	19.62 ± 0.2	23.02 ± 0.01	30.23 ± 0.23	35.05 ± 0.23
Gram (-ve)					
Escherichia coli	17.40 ± 0.01	20.21 ± 0.04	24.53 ± 0.02	32.11 ± 0.12	35.60 ± 0.02
Pseudomonus aeruginosa	17.90 ± 0.02	19.24 ± 0.01	24.25 ± 0.01	30.35 ± 0.18	34.83 ± 0.14
Shigella flexneri	17.40 ± 0.03	20.22 ± 0.03	24.23 ± 0.03	31.19 ± 0.21	35.62 ± 0.31
Shigella dysenteriae	18.11 ± 0.02	20.33 ± 0.05	24.22 ± 0.01	30.48 ± 0.21	36.14 ± 0.02
Shigella sonnei	17.51 ± 0.04	19.14 ± 0.05	23.24 ± 0.01	30.58 ± 0.11	35.51 ± 0.11
Shigella boydii	18.20 ± 0.02	20.12 ± 0.01	25.80 ± 0.53	32.43 ± 0.01	36.08 ± 0.01
Shigella shiga	18.20 ± 0.01	20.13 ± 0.02	25.42 ± 0.04	32.13 ± 0.31	36.13 ± 0.21
Salmonella typhi	17.60 ± 0.01	20.21 ± 0.02	25.20 ± 0.04	30.09 ± 0.03	34.97 ± 0.02
Salmonella paratyphi	17.50 ± 0.07	20.52 ± 0.06	24.32 ± 0.05	31.18 ± 0.03	35.51 ± 0.03
Vibrio mimicus	17.33 ± 0.03	19.32 ± 0.01	23.17 ± 0.07	31.44 ± 0.11	35.63 ± 0.18

The assay was performed in triplicate and the results are the mean of three values ± Standard Deviation.

Table 3: Minimum Inhibitory Concentrations (MICs) of three fractionate (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.) crude ethanol extract.

Bacterial strains tested	MIC values (µg/ml) of three fractionates			Ampicillin (30µg/disc)	Kanamycin (30µg/disc)
	PEF	EAF	CHF		
Gram (+ve)					
Staphylococcus aureus	256	128	64	16	8
Streptococcus agalactiae	256	128	64	8	4
Klebsiella pneumoniae	256	128	64	16	8
Streptococcus pyogenes	256	128	64	4	4
Bacillus cereus	256	128	64	8	8
Bacillus megaterium	256	128	64	8	4
Bacillus subtilis	256	128	64	16	16
Clostridium perfringens	256	128	64	4	8
Gram (-ve)					
Escherichia coli	128	64	32	8	2
Pseudomonus aeruginosa	128	128	64	16	8
Shigella flexneri	128	128	64	16	4
Shigella dysenteriae	256	128	64	16	8
Shigella sonnei	256	128	64	8	4
Shigella boydii	128	64	32	4	8
Shigella shiga	128	64	32	4	8
Salmonella typhi	128	64	32	4	2
Salmonella paratyphi	128	64	32	16	4
Vibrio mimicus	128	128	64	16	16

Table 4: Antifungal activities of three fractionate (PEF, EAF & CHF) of *Launaea sarmentosa* (Willd.) crude ethanol extract.

Fungal strains tested	Inhibition zone in diameter (mm) of three fractionates (500µg/disc)			Ketoconazole (20µg/disc)	Griseofulvin (20µg/disc)
	PEF	EAF	CHF		
Aspergillus niger	11.57 ± 0.04	14.17 ± 0.01	16.57 ± 0.07	21.22 ± 0.04	20.77 ± 0.05
Aspergillus ochraceus	12.13 ± 0.14	13.54 ± 0.11	14.73 ± 0.04	21.03 ± 0.11	21.18 ± 0.11
Aspergillus ustus	10.94 ± 0.01	14.34 ± 0.05	15.34 ± 0.01	22.04 ± 0.05	21.91 ± 0.07
Candida albicans	11.92 ± 0.22	14.22 ± 0.07	15.92 ± 0.02	21.32 ± 0.03	20.98 ± 0.22
Rizopus oryzae	12.08 ± 0.17	14.70 ± 0.04	16.08 ± 0.03	22.08 ± 0.17	22.28 ± 0.10
Trichophyton rubrum	10.68 ± 0.33	14.68 ± 0.02	14.98 ± 0.13	22.68 ± 0.33	22.38 ± 0.33

The assay was performed in triplicate and the results are the mean of three values ± Standard Deviation.

DISCUSSION

In recent time, bacteria and fungi have become the major life threatening pathogens in human being. Although, there are a large number of potent antibiotics available, development of resistance and multidrug resistance of the microorganisms to the current antibiotics are continuously emerging. The major causes are lack of proper knowledge of the people, inappropriate treatment facilities as well as misuse of antibiotics. Considering the raising levels of antibiotic resistance among pathogenic bacteria, the search for new, alternative and effective antibacterial agents has become a very important concern worldwide [41]. Actually, large parts of the world population rely on the traditional medicinal plants for the management of various illnesses ranging from simple fever to serious microbial infections. In this respect, plants provide a large reservoir of medicinally important constituents and especially their therapeutic properties may be used for the development of antibiotics [42]. Therefore, this study was focused to assess the presence of phytochemicals and antimicrobial activities of *Launaea sarmentosa* (Willd.) plant extracts. It is well known that the different classes of phytochemicals present in plant extracts are affected by sample preparation and extraction process and are responsible for their antimicrobial activities [41, 43]. Here, phytoconstituents were fractionated with different solvents according to varying polarities. Ethyl acetate and chloroform being highly polar solvents extracted most of the phytoconstituents; flavonoids, phenolic compounds, alkaloids, glycosides, steroids, saponins, tannins to a large extent (Table 1) and thereby exhibited significant antimicrobial activities (Table 2, 3, 4) compared to less polar petroleum ether. These findings corroborate with previous studies on *Launaea sarmentosa* (Willd.). Moghal, M. M. R., et al 2016 reported that the medicinal properties of this plant largely depend on several biomolecules such as tannins, flavonoids, alkaloids and phenolic compounds etc [44]. The current study revealed that among the three fractionates, the CHF exhibits greater antibacterial activity to gram (-ve) bacteria; *Shigella boydii*, *Shigella shiga, Salmonella typhi, Escherichia coli, Salmonella paratyphi, Pseudomonus aeruginosa*, etc with the highest inhibition zone (Table 2). This result agrees with the research reported by Millat, M. S., et al 2017. They found that *Launaea sarmentosa* (Willd.) crude methanolic extract showed good antibacterial activities against gram (-ve) bacteria and were resistant against gram (+ve) bacteria [45]. Unlike their results, the present study revealed appreciable sensitivity of the crude extract to gram (+ve) MDR strain; *Klebsiella pneumoniae*, *Staphylococcus* a*ureus* etc. Furthermore, the present study demonstrated that all fractionates exhibit varying MIC values and the lowest value belongs to CHF (32µg/ml) against gram (-ve) strains (Table 3). Moreover, due to the presence of bioactive compounds in large extent, the CHF inhibits the growth of pathogenic fungi like *Aspergillus niger*, *Rizopus oryzae*, *Candida albicans* etc more significantly than EAF and PEF. Therefore, considering the above results concisely, significant antibacterial activities, lowest MIC values, good antifungal activities as well as presence of potent biomolecules present in various fractionates of the crude extract, the plant *Launaea sarmentosa* (Willd.) may serve as a candidate for the development of an useful antimicrobial agent.

CONCLUSION

Multidrug-resistant (MDR) pathogens are an emerging threat to human. Previous reports recognized *Staphylococcus* a*ureus*, *Klebsiella pneumoniae, Escherichia coli* strains are MDR pathogens. Surprisingly, the present study made the plant *Launaea sarmentosa* (Willd.) much more important for its large number of potent phytochemical constituents, appreciable antibacterial activities to MDR strains, and good antifungal effect to pathogenic fungi. The present investigation is found to reveal more antibacterial than antifungal activities and among the three solvent fractionates the CHF produced the best results. Considering these findings, *Launaea sarmentosa* (Willd.) crude extract may serve as the lead for the discovery of new, safe and more efficient antimicrobial agents to fight MDR microbes. Further research is needed to isolate the bioactive compounds from the most active fractionate (CHF) of crude ethanolic extract having significant antimicrobial activities, mechanism lies in these activities and the cytotoxicity experiments to check for its safety.

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Conflicts Of Interest

The authors declare that they have no Conflict of interest.

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