



Screening of local medicinal plant extracts against multi drugs resistance bacteria

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Abstract: Multi Drugs Resistance (MDR) bacteria are mostly resistant to most of antibiotics, this leads to several severe infections and diseases. Thus the desire of new antibiotic sources are required which direct to the screening of new medicinal plants and use against MDR pathogenic bacteria. In our study, the antibacterial activity of three different plant extracts are utilized against pathogenic bacteria in-vitro to treat the infection and disease cause by pathogenic bacteria. The extracts were isolated from *Mallotus philippensis*, *Silybum marianum* and *Stachys parviflora* Benth in four different solvents extracts and were tested against eight pathogenic MDR bacterial strains (*Brucella abortus*, *Escherichia coli*, *Enterobacter sakazakii*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive) through well diffusion and disc diffusion. It was found that the extracts of selected plants showed maximum activity against all bacterial strains. The recorded zones of inhibition were 8.0-26.33mm for methanolic, 6.0-17.66mm for chloroform extracts and 8.01-2.33mm for ethyl acetate extracts. So, it is cleared from the results that the tested plant extracts have great potential as antibacterial compounds against bacteria. However, further research is required to isolate and identify the active ingredients vital for further pharmacological evaluation. Also screening of these plants for Anticancer and Anti-diabetic activity will be significant.

Key words: Antibacterial Activity; Medicinal Plants; Phytochemicals; MDR Bacteria; Zone of Inhibition.

Introduction

Human pathogenic bacteria are constantly considered as the main cause of disease and death. To treat diseases caused by bacteria, pharmaceutical industries are continuously developing drugs, to contest bacteria which are getting resistance against antibiotics producing a global issue [1]. This increase in resistance to antibiotics of bacterial strains, has led to the emergence of multi drugs resistance (MDR) bacterial strains [2]. The new generation antibiotics are more expensive with short period effect, which increase chance of disease onset and death rate [3]. The need of new sources, most effective and low cost tested antibiotics are required, which leads to searching for new sources (plants) with most effective antibacterial agents. Those effective components in plants can serve as a source and model for new synthetic antibiotics [4, 5]. More than 50% of newly developing synthetic drugs have natural product sources. The medicinal benefits of plants are due to phytochemicals present in them, having pharmacological actions on human body [6]. According to the world health organization (1999) traditional medicines continue to provide health treatment for over 80% population of the world particularly in the developing countries [7].

Mallotus philippensis belongs to the family Euphorbiaceae and its local name is kambila or kamala [8]. Its fruit contains phytochemicals such as rottlerin fixed oil 47.80%, mallotoxin 5.83-40%, oleic, kamalin, myristics, palmitic acid, stearic acid, crotoxigenin, tannins, octa cosanol, Oxalic acid, citric acid, homorottlerin, rottlerin, iso-rottlerin and rhmnoside [9, 10]. Many studies suggested that bark extract of *Mallotus philippensis* has natural antioxidants, phenolic compounds, tannins, antioxidant activity and antiradical activity [11]. Various parts of *Mallotus philippensis* are used for the treatment of bronchitis, diarrhea, diabetes, tap worm eye disease as an antifungal, jaundice, malaria, urogenital infection etc. It also comprises pesticidal, anti-microfilaria, hepatoprotective, and anti-oxidant properties [7, 12].

Silybum marianum belongs to the family Asteraceae and commonly known as Ladys thistle [13, 14]. The major active component present in *Silybum marianum* is flavonolignan in isomeric mixture, collectively called silymarin. Silymarin composed of silydianin, silybin, isosilybin and silychristin [15]. The major pharmaceutical activities of silymarin reported are anticancer, antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anti-depressant, digestive tonic, cardio protective,

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immune stimulatory, neuroprotective and a demulcent [14].

Stachys parviflora Benth belongs to the family Lamiaceae. Its local name is baggibuti, it is a persistent herb [16]. Most of investigations showed that the species related to this genus have a lot of phytochemical present like flavonoids, terpenoids, alkaloids, phenethyl alcohol glycosides, glucosides and saponins [17-19]. With addition of these chemical *stachy parviflora* contains polyphenols, diterpenoids, iridoids, essential oils, fatty acids and many other compounds [20, 21]. Many studies showed that this genus have a lot of pharmacological activities for examples antibacterial activity [22], anticancer [23], anti-nephritic [24], anti-inflammatory [25], antioxidant effects [26], and anti-anxiety [27].

Materials and Methods

Plant Materials

The plants (*Mallotus philippensis* L, *Silybum marianum* and *Stachys parviflora* Benth) were collected from district Dir lower KPK, and were identified from botanical garden of University of Malakand by plant taxonomist (Nazim Husain).

Extraction

The plant materials, leaves of *Stachys parviflora* Benth, flower capitulum of *Silybum marianum*, seed, fruit and granular hairy red substance of *Mallotus philippensis* L. were dried in shade and grounded under sterile condition. The different extracts were obtained by macerated 25 gram powdered materials of plants in 150 ml of 100 % pure solvents (i.e. Methanol, ethyl acetate, chloroform and water). And then were filtered by using fine cloth. The final extracts were concentrated and obtained by a rotary evaporator [28, 29]. A 100 mg/ml fraction were made.

Antibiotics Standards

We used four antibiotic standards for comparison with plant extracts and quality check of antibiotics. Which includes, Ciprofloxacin (5 µg), Cefepime (12 µg), Ceftriaxone (5 µg) and Moxifloxacin (12 µg). These synthetic antibiotics were purchase in medical store and the final solution was made in the laboratory.

Bacterial Strains

The MDR bacterial strains were obtained from Lady Reading Hospital (LRH) and identified by Department of Pathology, University of Peshawar. Bacterial strains were *Brucella abortus*, *E. coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Providencia stuartii*, *Pseudomonas aeruginosa* (gram negative) and *Staphylococcus aureus* (gram positive) isolated from the human blood and urine.

Antibacterial Assay of Plant Extracts

For bacterial growth and their sub-culturing, nutrient agar was used while disc diffusion and well diffusion methods were used to determined antibacterial activity of plant extracts [30]. In disc diffusion method, 5.0mm sterile paper discs with the plant extracts were then placed over the inoculated media in petri dishes. On the other hand, wells diffusion method with little modification was used [21]. The prepared extracts about 30 µl of each plant were added to each wells. After this the petri dishes were incubated for 24 hours at 37 °C. The same process was done for antibiotic standards for bacterial sensitivity test. The evaluation of antibacterial activity was done by measuring the diameter of zones of inhibition in mm against tested bacteria.

Results and Discussion

Antibacterial activity of *Mallotus philippensis*:

To determine the activity of *M. philippensis* we used 100 mg/ml fraction, which showed high zone of inhibitions. Table no 1 shows the results of 100 mg/ml fraction of *M. philippensis* extracts and antibiotic standards after 24 hours incubation. The seed (methanolic) extract showed poor activity against most of bacteria. The highest activity of seed (methanolic) extract was recorded against *E.coli* (8.3mm zone of inhibition), followed by *Brucella abortus* (8.0mm) and *P. stuartii* (8.0mm) and then *Proteus vulgaris* (7.6mm) *Staphylococcus aureus* (7.6mm). *P. aeruginosa* (6.0mm) *Enterobacter sakazakii* (7.0mm) and *Klebsiella pneumonia* (7.3mm). Another extract of Seed (chloroform) showed also poor activity against all tested bacteria such as 8-6.0mm inhibition zones for most bacteria. Granular fruit substance (methanolic) showed high activity against all tested gram negative bacteria, activity against *Proteus vulgaris* (18.6mm) and *P. aeruginosa* (18.3mm) was effective followed by *E. coli* (17.0mm), *P. stuartii* (16.3mm) *Enterobacter sakazakii* (15.3mm) *B. abortus* (14.3mm) and *S. aureus* (13.3mm). Granular fruit substance (aqueous) showed moderate activity against *K. pneumonia* (7.6mm zone of inhibition) followed by *E. coli* (9.6mm), *E. sakazakii* (10mm), *P. stuartii* (10.3mm), *S. aureus* (11.0mm), *B. abortus* (11.3mm), *Proteus vulgaris* (14.0mm) and *P. aeruginosa* (13.0mm).

Fruit extracts were used in three solvents. The fruit (methanolic) extract showed significant activity against all tested bacteria as shown in Table no.1. The activity against *Brucella abortus* (12.0mm), *P. aeruginosa* (13.3mm) *Enterobacter sakazakii* (16.6mm) was found moderate followed by *K. pneumonia* (16.0mm) *Proteus vulgaris* (15.3mm), *S. aureus* (14.0mm), *P. stuartii*(14.0mm) and *E. coli* (14.0mm). Fruit (chloroform) extract also showed effective activity against *Brucella abortus* (17.60mm) *S. aureus* (17.60mm), *K. pneumonia* (16.6mm) and *P. stuartii* (16.6mm). Activity against *E. coli*, *P. aeruginosa*, *E.*

sakazakii and *Proteus vulgaris* was recorded 17.0mm, 16.0mm, 15.6mm and 15.0mm zones of inhibition respectively. Fruit (Ethyl Acetate) extract showed poor activity against most of tested bacteria, such as *B. abortus* (9.3mm), *Proteus vulgaris* (9.8 mm), and *S. aureus* (9.6mm) followed by *E. coli* (8.60mm), *E. sakazakii* (8.60mm) and for *P. stuartii*, *K. pneumonia* and *P. stuartii*, it was 7.6mm zones of inhibition. In comparison, antibiotic standards showed higher activity against tested bacteria except cefepime (*B. abortus* 8.1mm).

In comparison, the antibiotic standards showed greater activity against most of tested bacteria except cefepime, which showed lower activity against *Brucella abortus* (8.1mm) and *Klebsiella pneumonia* (14.0mm), ciprofloxacin showed minimum activity against *E. coli* and *E. sakazakii* as compare to fruit (chloroform) extract, zones of inhibition were 14.2mm and 9.3mm respectively and ceftriaxone showed low activity against *E. sakazakii* (15.3mm) while moxifloxacin showed almost high activity against all tested bacteria.

Our study showed that seed (methanolic) extract has good activity against gram negative bacteria, which confirm the study of [31]. However, in case of *S. aureus* little contradiction was found. The study of Kumar [32], showed that *Mallotus philippensis* Granular red hairy substance (dichloromethane and methanol, 1:1, v/v) extract was no such activity against gram positive bacteria such as *S. aureus*, *Bacillus cereus varmycoides*, *Bacillus pumilus* and *Bacillus subtilis*, while having high activity against gram negative bacteria except *Klebsiella pneumoniae* (no activity).

While in our study, granular red hairy substances (methanolic and aqueous 100%) extract showed high activity against both gram positive and gram negative bacteria. The fruit extract showed excellent activity against gram positive and gram negative bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *S. aureus* which confirm the result of [33]. His study showed activity against *Pseudomonas aeruginosa* (22±0.52), *Escherichia coli* (18±0.30) and *Staphylococcus aureus* (18±0.27) inhibition zones for 60 mg/ml while our study showed activity against *Pseudomonas aeruginosa* (13.33mm), *Escherichia coli* (17.0mm) and *Staphylococcus aureus* (17.0mm) zone inhibition at 100 mg/ml (methanolic extract). The present and previous studies show that mallotus plant extracts has some special phytochemical which can stop and even kill bacteria. Thus it means that the phytochemicals are stronger and may be used as an antibiotic, these are rottlerin fixed oil, Mallotoxin, homorottlerin, rottlerin, isorottlerin, rhmnoside and many others [9, 10]. These extracts are also useful in other different treatments like bronchitis, diarrhea, and urogenital infections which are specially causes by bacteria [7, 11].

Antibacterial activity of *Silybum marianum*

Extract (flower Capitulum) of *Silybum marianum* was tested against eight bacteria as shown in Table no.2. Two different solvent extracts (methanolic and ethyl acetate) were used against selected bacteria, the results were measured after 24 hours, kept at 37 °C. The results of both extracts showed poor activity against most of bacteria. The flower Capitulum (ethyl acetate) extract showed moderate activity against some bacteria. such as *Brucella abortus* (10.3mm) and *P. stuartii* (10.3mm) *S. aureus* (12.8mm) while poor activity against *E.coli* (7.8mm) and there was no such activity showed against *P. aeruginosa* and *Proteus vulgaris*.

Another extract of flower Capitulum (methanolic) showed moderate activity against most of bacteria. Against *B. abortus* (12.3mm) *P. vulgaris* (12.3mm), *E. coli* (10.3mm), *K. pneumonia* (10.3mm), and *P. stuartii* (10.3mm) and for *E. sakazakii*, *P. aeruginosa* and *S. aureus* it was recorded 11.3mm, 10.6mm and 10.0mm zones of inhibition respectively. In comparison, ciprofloxacin showed lower activity against *E. sakazakii* (9.3mm) and cefepime also showed lower activity against *B. abortus* (8.1mm). Our results obtained from *Silybum marianum* flower blue capitulum (ethyl acetate and methanol) extracts showed activity against gram positive and gram negative bacterial strain, which confirm the results of [31], only in case of gram positive, which showed that *Silybum marianum* Capitulum extract was found very active against all Gram positive bacteria and demonstrated moderate to significant antibacterial activity against tested pathogens. Also our study confirms the result of [32], for *S. aureus*, while there was no activity recorded for *Silybum marianum* extract against gram negative bacteria except *Proteus vulgaris*. Most of previous studies suggest that the phytochemical compounds which are mostly present in *Silybum marianum* is silymarine highly active against both gram positive and gram negative bacteria, other compounds like silydianin, silybin, isosilybin and silychristin also present and all together called silymarine [15]. This compound is also useful in as an anticancer, antioxidant, hepatoprotective, anti-inflammatory and anti-depressant, digestive tonic [14].

Antibacterial activity of *Stachys parviflora* Benth

To determine the activity of *Stachys parviflora* Benth a fraction of 100 mg/ml fraction was used. Table no 2 shows the results of 100 mg/ml fraction of *Stachys parviflora* Benth leaves extracts and Antibiotic standards after 24 hours incubation. The highest activity against all tested pathogenic bacteria was shown by *Stachys parviflora* leaves (methanolic) extract. The activity against *P. vulgaris* (26.6mm), *E. sakazakii* (26.3mm), *P. aeruginosa* (26.3mm) *S. aureus* (26.3mm), *K. pneumonia* (25.6mm) *P. stuartii* (25.6mm) was more effective and activity against *E. coli* (24.0mm) and *B. abortus*

(24.3mm) also effective. In comparison, ciprofloxacin showed lower activity against most of bacterial strains, such as *E.coli* (14.2mm), *E. sakazakii*, (9.3mm), *P. aeruginosa* (22.1mm) and *S. aureus* (19.3mm), while cefepime showed lower activity as a whole except *p. aeruginosa* (34.9mm).

In case of moxifloxacin, it showed mostly higher activity against tested bacteria with few exceptions, such as *E.coli* (24.1mm) *E. sakazakii* (23.4mm), *P. stuartii* (21.5mm) and *S. aureus* (23.4mm). Ceftriaxone showed lower activity as compare to plant extract with few exception, such as *S. aureus* (33.3mm), while activity against *B. abortus* and *E.coli* was recorded 24.6mm and 24.5mm respectively. While activity against *P. stuartii* (22.5mm), *P. aeruginosa* (20.4mm) and *K. pneumonia* (20.3mm) was found lower.

The lowest activity was recorded against *E. sakazakii* (15.3mm) and *P. vulgaris* (19.0mm). In our study *Stachys parviflora* Benth showed maximum activity against both gram positive and gram negative bacteria, which confirm the results of the previous study of conducted in 2015 [34], showed that oil extracted from leaves of *Stachys parviflora* was active against *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus atrophaeus*, *Escherichia coli*, *Pseudomonas aeruginosa*. Some other study like by Kukic [26], of *Stachys* species have also shown antimicrobial activity in which zones of inhibition are 15-24mm. Thus eventually it is confirmed that this plant have vital compounds which are highly active against highly pathogenic bacteria. Different studies showed that this plant composed of lots of compounds like polyphenols, diterpenoids,

iridoids, essential oils, flavonoids, terpenoids, alkaloids, phenethyl alcohol glycosides and many more [18-21], These compounds are also used as an anticancer [23], anti-nephritic [24], anti-inflammatory [24], and antioxidant effects [26].

Conclusion

- This study showed that the tested medicinal plants have potential for excellent activity against both gram positive and gram negative bacteria.
- This concludes that these plants have the ability to use as antibiotics.
- This also offer a forward step towards the standardization of such plants as potential healthy source, which may be used in food and pharmaceutical industries.
- Furthermore, it would be of great interest if further research is carried out for the isolation of biologically active compounds present in the studied medicinal plants.

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Authors' contributions

Both authors contributed equally to this study.

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Table 1: Zone of Inhibition (mm) of *M. philippensis* against different bacterial strains after incubation of 24 hours at a concentration of 100mg/ml.

Bacterial Strains	Different Extracts of plant <i>Mallotus philippensis</i>											
	Seed Extract	Extract of Granular Red hairy substance on Fruit cover				Fruit Extract			Commercial Antibiotic Standards			
	Chloroform	Methanol	Methanol	Aqueous	Methanol	Chloroform	Ethyl acetate	Ciprofloxacin	Cefepime	Moxifloxacin	Ceftriaxone	
<i>B. abortus</i>	6.0	8.0	14.6	11.3	12	17.6	9.3	28.1	8.1	30.4	24.6	
<i>E. coli</i>	6.0	8.3	17	9.6	14	17	8.6	14.2	22.8	24.1	24.5	
<i>E. sakazakii</i>	6.0	7.0	15.3	10	16.6	15.4	8.0	9.3	16.1	23.4	15.3	
<i>K. pneumonia</i>	6.0	7.3	14.3	7.6	16	16.3	7.6	31.9	14	38.2	20.3	
<i>P. aeruginosa</i>	8.0	6.0	18.3	13	13.3	16	8.6	22.1	34.9	27.3	20.4	
<i>P. stuartii</i>	6.0	8.0	16.3	10.3	14.3	16.6	7.6	29.2	21	21.5	22.5	
<i>P. vulgaris</i>	6.0	7.6	18.6	14.0	15.3	15	9.6	28.4	22.1	32.2	19	
<i>S. aureus</i>	6.3	7.6	13.3	11.0	14.0	17.6	9.8	19.3	19.3	23.4	33.3	

Table 2: Zone of Inhibition (mm) of *Silybum marianum* and *Stachys parviflora* Benth against different bacterial strains after incubation of 24 hours at a concentration of 100mg/ml.

Bacterial Strains	Flower Capitulum Extracts of <i>Silybum marianum</i>		Leaves Extract of <i>Stachys parviflora</i> Benth	Commercial Antibiotics Standards			
	Ethylacetate	Methanol	Methanol	Ciprofloxacin	Cefepime	Moxifloxacin	Moxifloxacin
<i>B. abortus</i>	10.3	12.3	24.3	28.1	8.1	30.4	24.6
<i>E. coli</i>	7.8	10.3	24.0	14.2	22.8	24.1	24.5
<i>E. sakazakii</i>	9.6	11.3	26.3	9.3	16.1	23.4	15.3
<i>K. pneumonia</i>	8.6	10.3	25.6	31.9	14.0	38.2	20.3
<i>P. aeruginosa</i>	-----	10.6	26.3	22.1	34.9	27.3	20.4
<i>P. stuartii</i>	10.3	10.3	25.6	29.2	21.0	21.5	22.5
<i>P. vulgaris</i>	-----	12.3	26.6	28.4	22.1	32.2	19.0
<i>S. aureus</i>	12.8	10.0	26.3	19.3	19.3	23.4	33.3

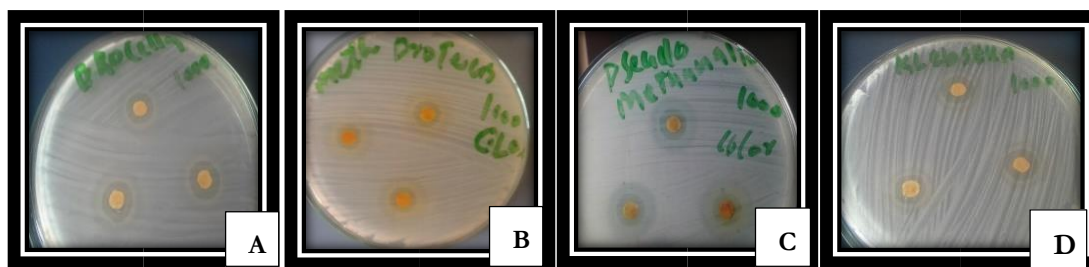


Figure 1: Extracts of *Mallotus philippensis* L. **A)** *B. abortus* (Chloroform Fruit). **B)** *P. vulgaris* (Methanolic Granular fruit Substance). **C)** *P. aeruginosa* (Methanolic Granular fruit Substance) & **D)** *K. pneumonia* (Chloroform Fruit).

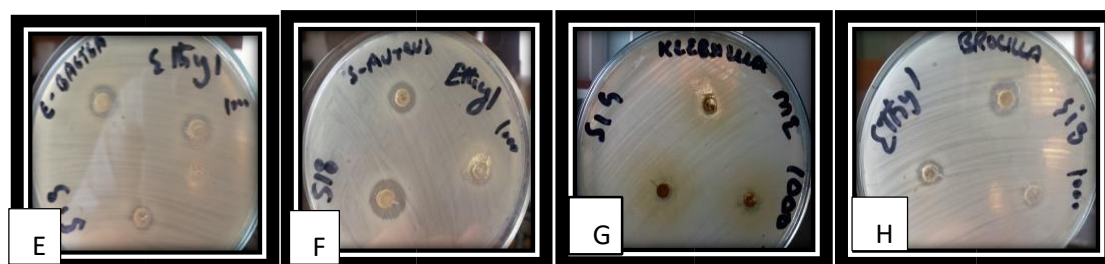


Figure 2: Extracts of *Silybum marianum* Blue Capitulum. **E)** *Enterobacter sakazakii* (Ethyl acetate). **F)** *S. aureus*. (Ethyl acetate) **G)** *K. pneumonia* (Methanol) & **H)** *B. abortus* (Ethyl acetate).

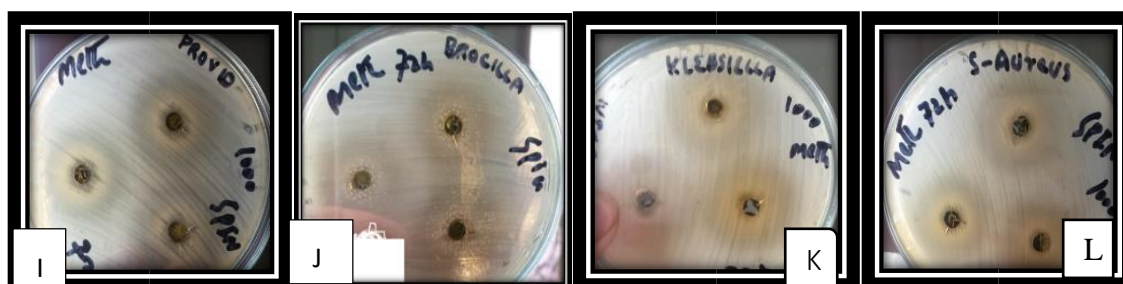


Figure 3: Extract of *Stachys parviflora* Benth leaves (methanolic) **I)** *P. stuartii*, **J)** *brocella abortus* **K)** *K. pneumonia*. & **L)** *S. aureus*.

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