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Antimicrobial Activity and Phytochemical Screening of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. leaves

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Abstract: The phytochemical screening of ethanolic extract of leaves of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. And their Antimicrobial activity were screened through by Disc diffusion assays against seven bacterial strains; (four Gram negative bacteria, three Gram positive bacteria) and one fungal strain *Candida albican*. Two concentrations (6 mg/ml and 12 mg/ml) were used to check the antimicrobial activity of plant extracts. The result showed that *Millotus philippensis* (Lam.) Mull. Arg leaves extract showed best activity against all tested microorganism at all concentration except *S. typhi,* while *Artemisia annua* L., were more active against gram positive bacteria but no activity was observed against *Salmonila typhi, Kleibsiella pneumonia* and fungal strain *Candida albican.* The phytochemical screening revealed the presence of secondary metabolites alkaliod, tanine, saponine and terpeniods in varying concentrations. This research supports the use of the leaves of Artemisia annua L and *Millotus philippensis* (Lam.) Mull. Arg for prophylactic and therapeutic purpose against bacterial and fungal infection.

Key words: Antimicrobial · Phytochemical screening · *Artemisia annua* · *Millotus philippensis*

INTRODUCTION

Many works have been done to knowing the different antimicrobial and phytochemical constituents of medicinal plants for the treatment of microbial infections [1]. During last century the practice of herbalism and great advances observed in modern medicine, make an important contribution to health care throughout the world [2]. This is due to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopoeias. Recent record of medicinal plants provides that 30% of worldwide drugs in the form of natural products isolated from them [3]. The medicinal value of plants is on the basis of some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, saponine and phenolic compounds [4], such as those used in protection against coronary heart disease and carcinogenesis [5]. *Artemisia* spp. has been reported to contain number of coumarins, flavones and terpenes [6]. *Artemisia annua* L. is an annual herb native of Asia and has been used for many centuries for the treatment of fever and malaria. This large genus has been the subject for numerous chemical studies such as alkaloid, Terpeniods, saponinine and phenolic substances [7]. *Millotus philippensis* (Lam.) Mull. Arg. is belong to family Euphorbiaceae, also locally called as Kamala a small tree grown on tropical region of south east Asia, Afghanistan and Australia [8]. *Millotus philippensis* (Lam.) Mull. Arg is small tree having small branches, green leaves flowering season is from June to November[9]. *Millotus philippensis* (Lam.) Mull. Arg. leaves highly medicinal plant due to the presence of secondary metabolites, for the treatment of various disease, like bronchitis problem, spleen enlargement and abdominal disease.

This study set out to investigate the antimicrobial activity and the secondary metabolites of *Artemisia annua* L and *Millotus philippensis* (Lam.) Mull. Arg leaves extract for the microbial infection and to search out the new antibiotic sources.

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MATERIALS AND METHODS

Plant Material: The fresh leaves of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. were collected from the different parts of District Malakand, Khyber Pukhtunkhawa, Pakistan. The plants specimens were identified with the help of taxonomists, Department of Botany, Hazara University, Mansehra.

Preparation of Extracts: Four hundred grams of each powdered air-dried plant leaves material was soaked in ethanol. The powders were regularly shaken for maximum extraction at 80rpm for 7 days. After 7 days, the extract was filtered using Whattman filter paper NO.1. The extracts solutions were evaporated to dryness under reduced pressure at temperature of 50°C using vacuum pump with the rotary evaporator.

Phytochemical Analysis: The freshly prepared leaves extracts were subjected to test the physiochemical screening for the presence of phytoconstituents,tannins. Saponins, Alkaliods, Terpenoids and sesquiterpenes [10]. To test for alkaloid about 0.2 g of the extract was warmed with 2% H_2SO_4 for few minutes. Then they were filtered and a few drops of dragendrof reagent were added. The orange red precipitate was observed for the presence of alkaloids. Extract 0.2g was dissolved in distilled water in test tube frothing shows the presence of saponin. For tannins a small quantity of each extract was mixed with water and heated on water bath. A few drops of ferric chloride were added. A dark green solution indicates the presence of tannins. For terpeniod 0.2 g extract was mix with 2ml chloroform and 3ml concentrated H_2SO_4 was added to form a layer. The formation of reddish brown coloration indicates the positive result for the presence of terpenoids.

This Is for Qualitative, but Whereis the Quatative Analysis for Each One ?

Test Microorganisms: Seven bacterial strains: three Gram positive: *Staphlyococus aureus, Bacillus subtilis* and *Bacillus atropoeus*, four Gram negative: *Escherichia coli, Pseudomonas aeruginosa, Salmonelatyphi* and *Kleibsiella pneumonia* and one fungal strain *Candida albicans* were used in this study. All the bacterial strains were clinical isolates obtained from the Pakistan Council of Scientific and Industrial Research (PCSIR), Peshawar,

Standard Drug: Three antibiotics were used in the experiment, azithromycin (50 µg/ml) for gram positive bacteria, ciprofloxacin (50 µg/ml) for gram negative and clotrimezole (50 µg/ml) for fungal strain.

Stock Solution: 10 mg of extract was taken and dissolved in 1 ml of dimethyl sulphoxide (DMSO), which was used as stock solution.

Culture Media: Nutrient agar media and nutrient broth media were used as a growth media for bacteria. The Media was composed of Agar 15.0g, peptone 5.0g and beef extract 3.0g. One liter media was prepared by dissolving 40g of nutrient agar in 700ml of distilled water. The pH was adjusted to 7.0 at 25°C.

Antibacterial Activity: The antibacterial activities of the extracts were observed by disc diffusion method. About 20 ml of molten nutrient agar was poured into the sterile petri dishes and allowed to set. About 50 µl of a 24 h old culture of each test organism was inoculated into the nutrient agar plate by sterile pipette. 12 µl and 6 µl of extracts were applied to the sterile perforated filter paper disc and placed on the nutrient agar plates seeded with the test organisms. Antibiotics azithromycin for gram positive strains and ciprofloxacin for gram negative strains were used as standard drugs. The plates were then incubated at 37°C for 24 h and the zone of inhibitions were measured.

Antifungal Activity: Suspension of microorganisms were added to sterile sabouraud dextrose agar medium at 45°C and the mixture was transferred to sterile petridishes and allowed to solidify. Sterile discs dipped in various concentrations (12 mg/ml and 6 mg/ml) of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg and clotrimezole (50 µg/ml) were placed on the surface of agar plates. The plates were left for 1h at room temperature and incubated at 37°C for 24 h. The diameter of zone of inhibition of extracts and standard was measured.

Statistical Analysis: All the tests were conducted in triplicates. The data were statistically analyzed and expressed as mean ± S.D.

RESULTS

Phytochemical constituents present in the plant leaves extract of *Artemisia annua* L., include alkaloids, tannins, terpeniods and saponins, while in leaves extract of *Millotus philippensis* (Lam.) Mull. Arg showed no record of alkaloids (Table 1). The result show that the leaves extract of *Artemisia annua* L., were more rich in phytochemical constituent as compared to the leaves extract of *Millotus philippensis* (Lam.) Mull. Arg. The leaves extract of *Artemisia annua* L., showed effective activity against most of the tested

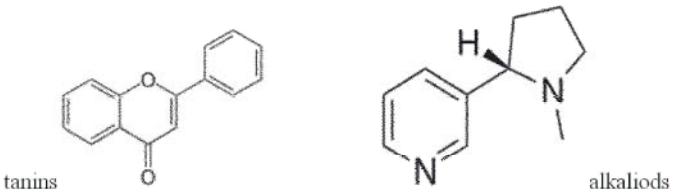

Fig. 1: Chemical structure of isolated alkaloid and tannins from the leaves of *Artemisia annua* L.

Table 1: Phytochemical in *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. leaves extracts.

	Phytochemical analysis	
Secondary metabolites	*Artemisia annua* L. leaves	*Millotus philippensis* leaves
Alkaloids	+ +	_ _
Tannins	+ +	+ +
Saponins	+ +	_ _
Terpenoids	+	+

Keys: ++ present in appreciable quantity, + present in low quantity, _ negligible

Table 2: Antibacterial Activity of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg.Ethanol Extract Against Gram negative Bacteria

	Diameter of zone of inhibition			
	Millotus philippensis		Antibiotics	
Artemisia annua L.,				
Bacterial strain	Concentration	leaves extract	leaves extract	Ciprofloxacin (50 µg/ml)
E. coli	6 mg/ml	16.4±0.2	0.0±0.0	43.2±0.8
	12 mg/ml	18.2÷0.2	0.0±0.0	
P. aeruginosa	6 mg/ml	13.0±0.4	12.4±0.3	42.4±0.0
	12 mg/ml	15.1÷0.2	15.3±0.3	
S. typhi	6 mg/ml	0.0±0.0	14.2±0.2	34.0±0.0
	12 mg/ml	0.0±0.0	16.2±0.2	
K. pneumonia	6 mg/ml	0.0±0.0	18.2±0.2	29.0±0.0
	12 mg/ml	0.0±0.0	23.1±0.3	

Table 3: Antibacterial Activity of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. Ethanol Extract Against Gram positive Bacteria

	Diameter of Zone of inhibition			
	Millotus philippensis		Antibiotics	
Artemisia annua L.,				
Bacterial strain	Concentration	leaves extract	leaves extract	Azithromycin (50 µg/ml)
S. aureus	6 mg/ml	17.5±0.4	20.3±0.2	23.0±0.0
	12 mg/ml	22.0÷0.5	21.8±0.7	
B. subtilis	6 mg/ml	23.3÷0.2	19.1±0.2	25.0±0.0
	12 mg/m l	15.1÷0.2	15.3±0.3	
B. atropoeus	6 mg/ml	17.0±0.1	17.2±0.2	30.1±0.1
	12 mg/ml	20.1÷0.1	20.1±0.2	

microorganisms at 12 mg/ml and 6 mg/ml concentrations. The highest activity was observed against *Staphlyococus aureus, Bacillus subtilis* and *Bacillus atropoeus*, *Escherichia coli, Pseudomonas aeruginosa,* while no activity was observed against *Salmonila typhi, Kleibsiella pneumonia* and one fungal strain *Candida albicans* (Table 2, 3).

The leaves extract of *Millotus philippensis* (Lam.) Mull. Arg show highest activity against *Staphlyococus aureus* and *Kleibsiella pneumonia* at all concentration. Low activity was observed against *Bacillus subtilis, Bacillus atropoeus*, *Escherichia coli, Pseudomonas aeruginosa,* while no activity was observed against *Salmonila typhi.* leaves extract of *Artemisia annua* L., were effective activity against tested microorganism as compared to leaves extract of *Millotus philippensis* (Lam.) Mull. Arg.

Table 4: Antibacterial Activity of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg.Ethanol Extract Against fungal strain *Candida albican*

	Diameter of Zone of inhibition			
	Millotus philippensis		Antibiotics	
Artemisia annua L.,				
Fungal strain	Concentration	leaves extract	leaves extract	Clotrimezole (50 µg/ml)
C.albican	6 mg/ml	0.0±0.0	11.1±0.5	32.4±0.5
	12 mg/ml	0.0±0.0	19.4±0.2	

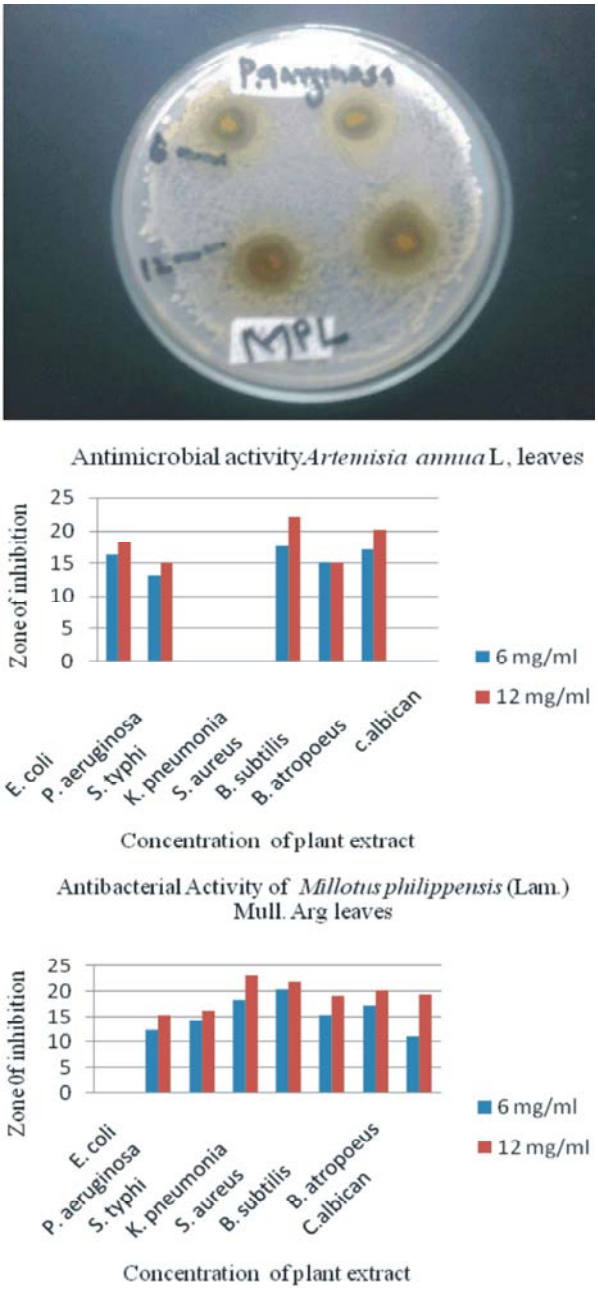

Fig. 2: Zone of inhibition (mm) of *Millotus philippensis* (Lam.) Mull. Arg. Leaves Ethanolic Extract Against *P.aeruginosa*.

DISCUSSION

Phytochemical constituents such as tannins, saponins, alkaloids and terpenoids secondary metabolites of plants that serve as defense mechanisms against predation by many microorganisms, insects and other herbivores [11, 12]. This can indicate the demonstration of antimicrobial activity by the leaves extracts of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg. The secondary metabolite are known to be biologically active against both Gram positive and Gram negative bacteria [13]. In this study the result showed that the leaves extract of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull. Arg have potential of antimicrobial activity due to presence of secondary metabolites. *Artemisia annua* L., leaves extract show best inhibatory activity against Gram positive bacteria: *Staphylococcus aureus, Bacillus subtilis* and *Bacillus atropoeus* while low zone of inhibition was scored against *Escherichia coli, Pseudomonas aeruginosa* no activity was observed against *Salmonila typhi, Kleibsiella pneumonia* and one fungal strain *Candida albicans* (Table 4). On the contrary observed that ethanol and methanol extract did not have activity on Gram positive and Gram negative bacteria [14]. Apart from antimicrobial activity these plant extract are also use for therapeutic purposes to cure several disorder [15]. *Millotus philippensis* (Lam.) Mull. Arg. leaves extract showed best zone of inhibition against all tested microorganism at all concentration except *S. typhi* [16] The result of the present study investigate that antimicrobial activity vary with the species and plant material used. Thus the certain the value of these medicinal plants which could be considerable interest to the development of new drugs.

CONCLUSION

The demonstration of antimicrobial activity and phytochemical screening of leaves extracts of *Artemisia annua* L. and *Millotus philippensis* (Lam.) Mull Arg may help to discover new classes of chemical antibiotics that

can serves selective agents for infectious disease. Further investigation to isolate the specific active constituents need to be carried out.

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