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PHYTOCHEMICAL STUDY AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS AND ITS DERIVED FRACTIONS OBTAINED FROM *BERBERIS VULGARIS* L. AND *STELLARIA MEDIA* L. LEAVES

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Abstract

This study was carried out with an objective to investigate the antibacterial and antifungal potential of leaves of *Stellaria media* Linn. and *Berberis vulgaris* Linn. In the present study, the antimicrobial activities of extracts and its derived fractions obtained from the leaves of *B. vulgaris,* and *S. media* were evaluated against pathogens by using agar well diffusion method. The extracts and fractions (10 µg/ml) of selected plants were tested against single Gram-positive bacterium Staphylococcus aureus, six Gram-negative bacteria *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Shigella dysenteriae* and three fungal pathogens *Fusarium solani, Alternaria alternata, Candida albicans*. Zone of inhibition of extracts/fractions were compared with the standards drug used streptomycin for antibacterial activity and nystatin for antifungal activity. The results showed that crude extract and fractions of S. media leaves gave good inhibitions ranging from 15 mm to 17.5 mm against tested bacteria and zone of inhibitions ranging from 6.8 mm to 16.5 mm against fungal pathogens. While extracts/fractions derived from the leaves of our second selected plant *B. vulgaris* showed no remarkable action against all tested microbes except *P. aeruginosa* against which plant showed good activities. The phytochemical investigation of our research plants showed that alkaloids, flavonoids, saponins, phenol, terpenoids and glycosides groups of chemicals are present in our selected plants. The antimicrobial activities can be correlated with the phytochemicals present in our research plants. Hence, these plants can be used to discover bioactive natural products; all these preliminary reports form a primary platform for further phytochemical and pharmacological studies.

Key words: Medicinal plants, *Berberis vulgaris, Stelleria media*, Antimicrobial activities, Antifungal activities. Phytochemical screening, Drug sighting.

Introduction

Medicinal plants and their extracts have exceptional attention because of their important influence on human health. Medicinal plants represent the primary source of the health care (Ikram *et al*., 2015). According to the World Health Organization (WHO) report, almost 80% of people in marginal communities use medicinal plants for the treatment of various diseases and illness (Calixto, 2000). Plants usually contain a variety of compounds like alkaloids, flavonoids, quinine, tannins and terpenoids that are effective agents against diseases (Mazandarani e*t al*., 2013; Habiba *et al.*, 2016). During the past few years, several studies have been focused on the medicinal evaluation of plants used in traditional medicine. These include examples of *Bonafousia* species, and *Heisteria acuminate* which possess anti-inflammatory activity and are commonly used in pathologies related to inflammation (Ortega *et al*., 1996). *Allium sativum* not only possesses anti-thrombogenic activity but also contains antibacterial, antifungal, and anticancer activity (Khan & Basar, 2017). *Berberis vulgaris* has been claimed to exert curative effects in the diseases caused by Salmonella species (Farhadi & Gavadifar, 2008). In past, barberry fruits have been used in the traditional medicine internally as tea and syrup for treatment of disorders of the respiratory tract, fever, infections, cold, and flu (Haq, 1997). The root and stem of these plants are also used for the diseases and disorders of the gastrointestinal tract, liver, gall bladder, kidney and urinary tract, respiratory tract, heart, and circulatory systems (Pieroni, *et al*., 2002). This plant is very common in Rawalpindi and Islamabad and a very little work has been done yet. It is commonly used in traditional and homeopathic medicines. The active ingredients in *Stelleria media* (seed), has antioxidant properties *In vitro* (Kumar *et al*., 2011). In Pakistan and other third world countries where infectious diseases are prevalent, there is a need to develop some medicines of plant origin against these persisting infectious diseases, which may be comparable to modern medicines and antibiotics (Tanne, 2006; Khan & Shinwari, 2016; Tariq *et al*., 2016). Because of the possible adverse side effects of synthetic drugs there is a need to look for the new natural alternatives (Najeebullah *et al*., 2021).

Materials and Method

Sample collection: The fresh leaves of selected plants *Stelleria media* and *Berberis vulgaris* were collected from District Hangu and were brought to the laboratory for further processing.

Rinsing the sample, drying, and crushing of leaves: After collection, the leaves were rinsed with water to remove the adherents. In addition to this, the leaves were further rinsed with ethanol to clean the samples properly and to protect it from infection's attack. After that the next work was to cut and break the leaves into tiny parts by

scissors. As the breaking and cutting process completed, the leaves were allowed for shade drying. After draying powder was made from leaves of selected plants, then the powder was kept in airtight bottles for further use.

Extraction and Fractionation: The following method was adopted for plant extracts. After plant grinding process, *Berberis vulgaris L.* powder were soaked in methanol for 10 days (Chen *et al.*, 2003). This practice was repeated three times and then the extraction process was performed. Similarly, after that the filtering of these plants extract was carried out. The remaining debris and impurities were separated, and the refining materials were got in a bottle (Sasidharan *et al*., 2011). The thick fluidly mixture obtained which was given to rotary evaporator for separation of solvent i.e. methanol. After complete evaporation solidified methanolic extract was got in a bottle that is airtight. Distilled water was then mixed to crude extract (sample) before fractionations. The solvent - solvent partitioned was done orderly with the solvent in the way as less to more polar wise such as (solvent *n*-hexane, chloroform, ethyl acetate and similarly one by one solvent *n*-butanol to get *n*-hexane soluble sample, chloroform soluble fraction, ethyl acetate-soluble and the last sample *n*- butanol soluble respectively. The same practice and method were done for *Stelleria media L.* to obtain the first sample that is crude after separation of methanol and other its fractions were given to phytochemical study and its antimicrobial activities.

The Method used in the work for anti-microbial effects: Standard protocols in Agar Well Diffusion (AWD) method were used to assess antimicrobial profile of selected plants B. *vulgaris* and *S. media* leaves (Gupta *et al.*, 2008).

Results and Discussion

Evaluation of antibacterial and antifungal activities of the present study revealed that the *S. media* possess potential antibacterial and antifungal activities against seven bacterial strains and three fungal species while *B. vulgaris* showed no remarkable activities against the selected bacterial and fungal species. From our research it is summarized that *S. media* crude extract and chloroform fractions inhibited the growth of the colonies of good number of bacterial and fungal species than the other solvent fractions studied.

Antimicrobial effects of leaf extract of *Stelleria media*: Among the five solvent fractions, the chloroform fraction showed higher inhibitory activity that is 17.5 mm and crude extract 14.5 mm against the bacterial, *Shigella dysenteriae* and 16.5 mm and13.2 mm against the fungal, *Fusarium solani* respectively. The chloroform fraction and crude extract showed significant activity against all the tested bacterial and fungal species which was ranging between 8 mm, 10 mm, 14 mm and 17 mm respectively. However, the ethyl acetate and n-hexane fractions showed moderate activity against the bacterial and fungal specie (Figs.1, 2).

Crude extracts and its derived fractions from *B. vulgaris* showed no inhibition against all tested bacterial strains except *P. aeruginosa* against which the plant showed good inhibitory activity that is crude extract 16 mm, *n*-hexane 13.5 mm, chloroform 14 mm, E. acetate 12.5 mm, and *n*-butanol 12.5 mm (Fig. 3). Antifungal result showed that *B. vulgaris* gave low inhibition activity against tested fungi except *C. albicans,* ranging from 10 mm, 16 mm and 21 mm respectively (Fig. 4).

Phytochemical analysis of selected plants: It is discovered from the present investigation that the leaves extract of selected medicinal plants contained some major compounds listed below in the table. However, alkaloid and flavonoid were only detected absence (-ive) in methanolic extract of *Stellera media* plant while in extract of *Berberis vulgaris* leaf, alkaloid was found abundantly presence (+++) (Tables 1, 2).

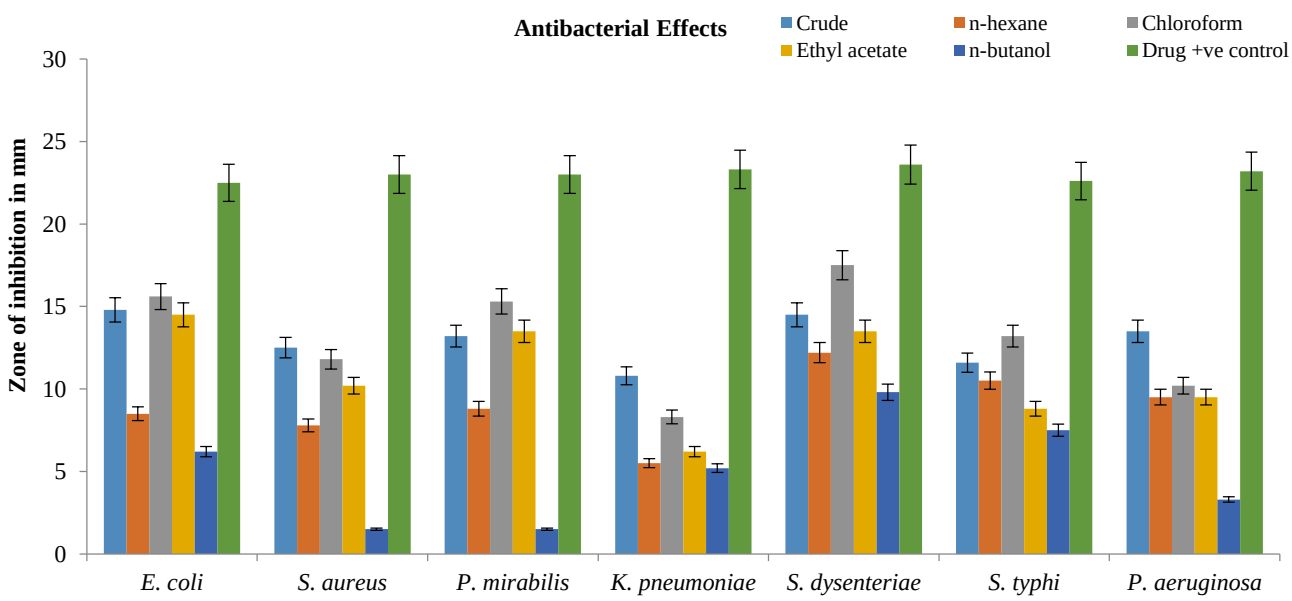

Fig. 1. Antibacterial effects of leaf extract / fraction obtained from Stellaria media.

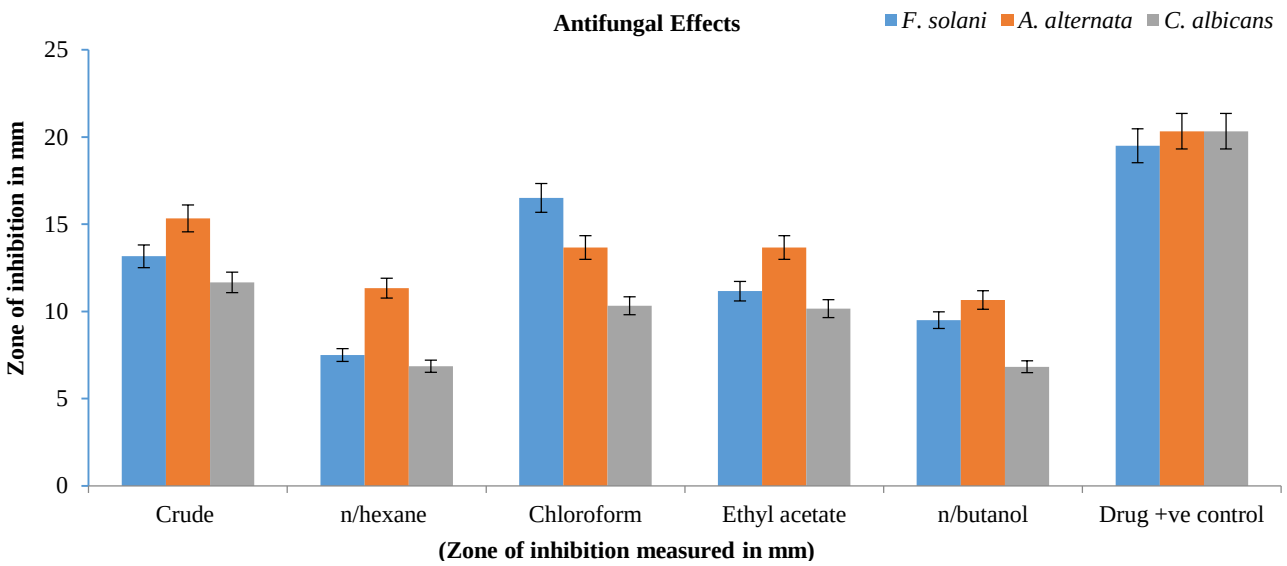

Fig. 2. Antifungal effects of leaf extract / fraction obtained from *Stellaria media*.

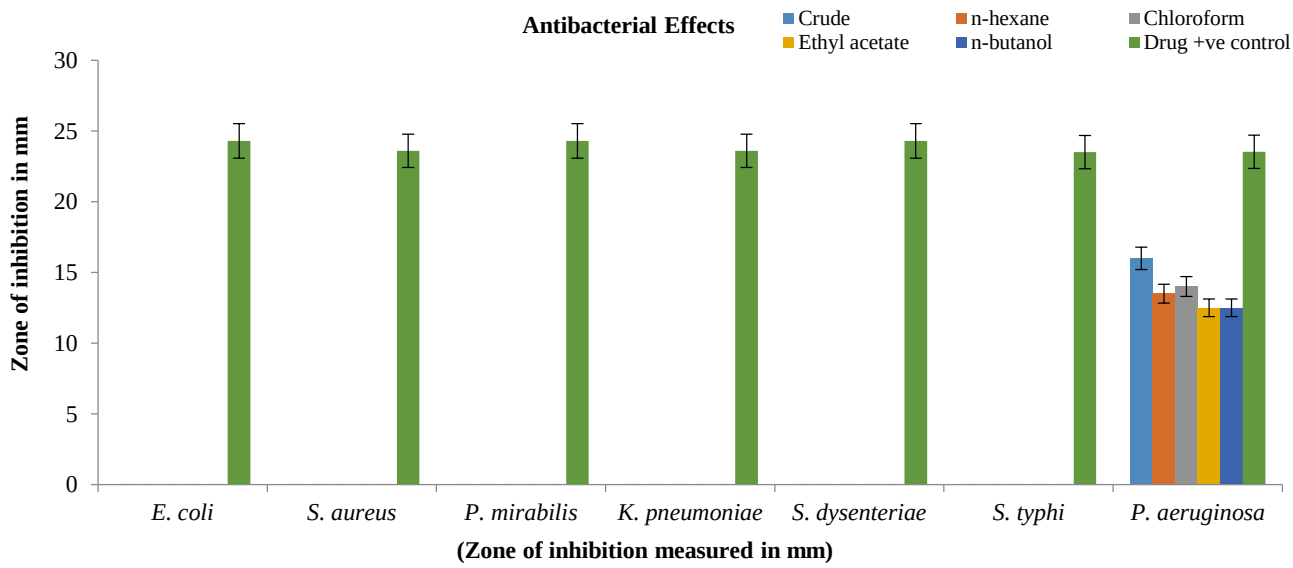

Fig. 3. Antibacterial effects of leaf extract / fraction obtained from *Berberis vulgaris*.

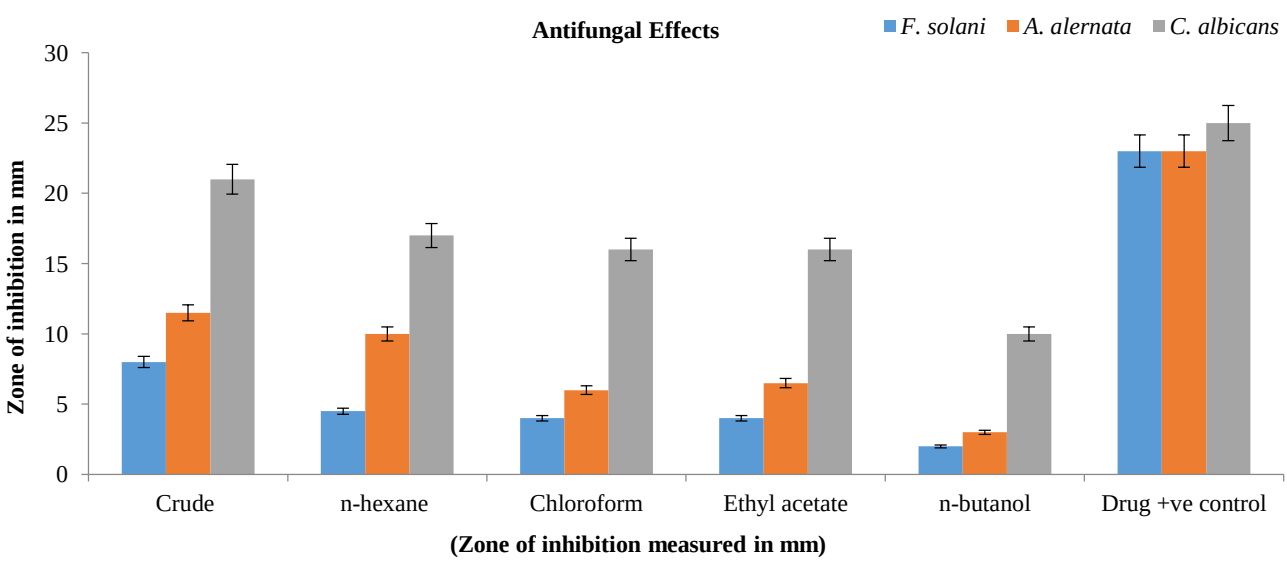

Fig. 4. Antifungal effects of leaf extract / fraction obtained from *Berberis vulgaris*.

Table 1. Preliminary phytochemical screening of *Stelleria media*.

S. name of flora	Major compounds	Abundantly presence	Normally presence	Slightly presence	Absence
Stelleria media L.	Alkaloid				-
	Saponin			+	
	Flavonoid		++		
	Glycosides				-
	Phenol		++		
	Terpenoid				-
	F. acid		++		

Table 2. Preliminary Phytochemical Screening of *Berberis vulgaris.*

S. name of flora	Major comounds	Abundantly presence	Normally presence	Slightly presence	Absence
Berberis vulgaris L.	Alkaloid	+++			
	Saponin			+	
	Flavonoid		++		
	Glycoside				-
	Phenol	+++			
	Terpenoid				-
	F. acid			+	

Discussion

The leaf extracts/fractions of S. media showed significant and remarkable inhibitory activity against all selected/tested bacterial species, of these extract/fractions the highest mean zones of inhibitions were seen against all above mentioned species are crude extract and chloroform fractions as shown in figure. The crude extract result was examined that is 14.8 mm showed the highest inhibitory action against *E. coli* and gave 10.8 mm revealed the lowest mean inhibitory activity against *K. pneumonia*. This result agrees with the view of (Maharjan *et al*., 2011 and Hiremath *et al.*, 2011; Shinwari *et al.*, 2020) selected the same bacterial strains like in our research work presenting good and significant effects in response of these all above mentioned bacteria species whereas low anti-bacterial effects given in response of *S. auraus.* Evaluation of antibacterial and antifungal activities of the present study revealed that the *S. media* possess remarkable potential antibacterial and antifungal activities against seven bacterial strains and three fungal species. These finding strongly agree with the *Azadirachta indica* plant leaves having good anti-fungal activity, showing the great potential of major natural compounds and is good effective in the terms of primary health treatment (Anil & Sushil, 2000). The plant extracts of Neem also having significant and useful as natural drug, could be effective against infections causing harmful microorganism. The following phytochemicals also been reported such as alkaloids, glycosides, flavonoids and saponin. These are used as antibiotic and great potential of these plants against different disease-causing pathogens (Koona & Budida, 2011; Ayatollahi *et al*., 2019). This previous literature is also correlated with our findings of antifungal activity of leaves extract / fraction of *S. media* and *B. vulgaris*. *B. vulgaris* showed no remarkable activities against the selected bacterial and fungal species. From our research it is summarized that *S. media* crude extract and chloroform fractions inhibited the growth of the colonies of large number of bacterial and fungal species than the other solvent fractions studied. This result is agreement to (Gupta *et al.*, 2008) in laboratory, the antifungal activity of berberine was also evaluated as antimicrobial activity against C. albicans. Their inhibition zones showed good and reasonable effects as natural drug. According to (Koona & Budida, 2011), reported that the extraction/fractions of some medicinal plants having good agents showed antimicrobial activities against all tested microorganisms especially against *C. albicans* whereas in case of our study it was examined that the leaf extract / fraction of S. media showed good and significant activity against all tested fungi including *C. albicans* that is 11.6 mm highest mean inhibitory action and antifungal result for selected second medicinal plant B. vulgaris gave low inhibition activity against tested fungi except *C. albicans,* ranging from 10mm, 16mm and 21mm respectively. This indicates that the leaves extract and fraction of S. media and *B. vulgaris* showed good and significant activity and inhibited the growth and multiplication of bacteria and fungi species. This inhibitory effect could be attributed principally to bioactive drug development as well as biological control pathway on the bacteria and fungi diseases/infections.

Conclusion

The present study revealed that the leaves extract/ fraction of *Stellaria media* possess potential antibacterial and antifungal activities. It is interesting to note that the chloroform and crude extracts of *Stelleria media* showed

highest inhibition zones that is 17.83 mm and 14.16 mm against *Shigella dysenteriae.* In case of *Berberis vulgaris* no remarkable antibacterial and antifungal activities were observed against tested bacterial and fungal species.

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