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Effectiveness of traditional medicinal herbs against pathogens causing wide spread diseases

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ABSTRACT

The complications due to microbes are in the rise in developing countries especially in the remote areas due to non-availability of proper treatment. In this regards, people are compelled to use traditional herbs for treatments. In the present work the antimicrobial activity of three medicinal herbs, *Foeniculum vulgare*, *Curcuma longa* and *Berberis vulgaris* were studied for the actions against wide spread Gram-negative bacterial and fungal strains, *Escherichia coli, Salmonella typhi, Mycoplasma gallisepticum* and *Shigella dysenteriae* and four fungal strains, *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Fusarium solani* by agar well diffusion method. The study shows that *n*-hexane and chloroform extracts of *F. vulgare* exhibited significant antibacterial activity against *S. dysenteriae* and *M. gallisepticum* while the crude and *n*-hexane fractions of *C. longa* exhibited promising antibacterial affect against *E. coli* and *S. typhi*, respectively. Ethyl acetate fraction of *Berberis vulgaris* showed good activity against *M. gallisepticum,* whereas Ethyl acetate fraction of *C. longa* showed the best activity against *Aspergillus fumigates* and *A. niger*. The study confirms the effectiveness of these medicinal plants and its different extract against the common diseases caused by *E. coli*, *S. typhi, M. gallisepticum*, *S. dysenteriae*, *A. flavus, A. fumigatus*, *A. niger* and *F. solani*.

INTRODUCTION

Traditional medicine has been a prime source of cure in the third world and the trend of using traditional medicine is on the rise all over the world due to its wide range of efficacy and minimum side effects. Herbs are the main source of providing the therapeutic agents in traditional medicine, because the plants have the potential to synthesize a variety of chemical entities that on one hand defend them against attack from predators such as insects, fungi and herbivorous mammal and on the other hand perform various important biological functions and protection against diseases in human being. Many of these phytochemicals like Flavonoids, alkaloids, tannins and phenolic compounds are established as the most prominent bioactive plant metabolites. They had proved lethal against pathogens causing diseases and have long-term health beneficial effects when consumed by humans (Ullah et al., 2013). Medicinal plants are economically important due to a good earning source for the local population and considerably useful in the treatment of a number of ailments (Muslim et al., 2012). Some of these plants and herbs or their portions are used in daily life in different food recipes. These medicinal plants are used against different pathogens in raw form and as extracts. It is well known that even the most synthetic drugs have their origin from plant products (Malarkodi and Manoharan, 2013). The following three plants namely *F. vulgare*, *C. longa* and *B.vulgaris* were selected for this Purpose.

F. vulgare a flowering plant belongs to the family *Apiaceae* commonly called *carrot* family. The common name of the specie is fennel, reputed for its medicinal and aromatic properties. In folk medicine the plant is

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used as carminative, digestive, lactogogue, diuretic and also in treating respiratory and gastrointestinal disorders (Shahat et al., 2011).

C. longa belongs to family *Zingiberaceae* (ginger), a perennial herb, pointed leaves and funnel-shaped yellow flowers. The rhizome is the active part of the plant used as food ingredient with medicinal properties. Dried *C. longa* rhizome is the source of the spice turmeric, the ingredient that gives curry powder its characteristic yellow color. Turmeric is used extensively in foods for its flavor and color, as well as having a long tradition of use in the Chinese and Ayurvedic systems of medicine, particularly as an anti-inflammatory and for the treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage and colic. Turmeric can also be applied topically in poultices to relieve pain and inflammation (Ikhlas and Khan, 2010). *C. longa* Linn.is known to possess various therapeutic activities and has been used by medical practitioners as an anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrheal, hepatoprotective, anti-asthmatic and anti-cancerous drug. It is also widely used in cosmetology (Vasavda et al., 2013).

Barberry (*B. vulgaris* Linn.) belongs to family *Berberidaceae*. It is found in Pakistan, India and other tropical regions of the world. In folk medicine different parts of the plant are effectively used as analgesic, anticoagulants, to treat gastric ulcer, diarrhea and treating scurvy (Rahimi-Madiseh et al., 2017).

Looking to the enormous uses of the selected plants, the plants were collected for the scientific exploration of their medicinal properties. The aim of the study was to investigate the antibacterial and antifungal activity of *F. vulgare*, *C. longa* and *B.vulgaris* and authenticate there uses in the folk medicine.

MATERIALS AND METHODS

Plant materials

The samples of *F. vulgare*, *C. longa* and *B.vulgaris* were collected from District Karak, Khyber Pakhtunkhwa, Pakistan. The Samples were authenticated by plant taxonomist at Department of Botany, KohatUniversity of Science & Technology, Kohat.

Test microorganisms for antimicrobial assay

Bacterial culture of *E. coli*, *S. typhi*, *M. gallisepticum*, *S. dysenteriae* and fungal strains *A. niger*, *A fumigates*, *A. flavus* and *F. solani*were provided by the culture collection centre, Department of Microbiology, Kust, Kohat and Department of Microbiology, University of Agriculture Peshawar, Khyber Pakhtunkhwa, Pakistan. The bacteria were maintained on nutrient broth at 37°C and fungi were stored on potato dextrose agar (PDA) at 28°C.

Extract preparation

The plant material was cleaned and washed with water and then dried in open air. The dried material was chopped into pieces and soaked in methyl alcohol for two weeks. The solvent of the extract was removed under reduced pressure using rotary evaporator and then dried. The dried extract was suspended in water and then extracted with Hexane, Chloroform and Ethylacetate to get solvent soluble fractionations (Ahmed et al., 2009). The crude extract and its various solvent soluble fractions were tightly packed and stored at 4°C.

Antibacterial activity

To determine the antibacterial activities of different solvent soluble fractions of *F. vulgare*, *C. longa* and *B. vulgaris* were used against bacterial strains like *E. coli*, *S. typhi*, *M. gallisepticum* and *S. dysenteriae* by modified agar well diffusion method (Umesh and Hindumaty, 2012). Solutions of crude extract and its different fractions at concentration of 3 mg/mL were prepared in dimethyl sulfoxide (DMSO).

The media for the antibacterial activity was prepared by dissolving 14 g nutrient agar in double distilled water in a 500ml flask. The media was sterilized in an autoclave at high pressure for 15 minutes at 121°C along with Petri dishes, cork borer and pipette. The media was then transferred into Petri dishes under laminar flow hood. In the agar media wells of 7mm were bored using the sterile cork borer, and then inoculated the bacterial strain culture at a concentration of 10^6 CFU/mL into the wells made in the nutrient agar. These inoculated wells were then added the already prepared stock solution of the crude and fractionated extracts. As a control azithromycin and ciprofloxacin antibiotics solution were added to separate wells while as a negative control DMSO was added to other separate well. These inoculated Petri dishes were then incubated at 37°C for 24 h. After 24 h incubation antibacterial activities were measured by measuring the diameter of the zones of inhibition and were compared with the zone of inhibition of standard drug. The amount of growth in each well was measured in mm (Ramirez et al., 2006).

Antifungal activity

The antifungal assay was performed using disc diffusion method. For the activities nutrient broth was used as a

medium for developing the culture of fungal strains. The media was prepared according to the manufacture specification dissolved the specified quantity of dry powder of nutrient agar in a given volume of distilled water and then autoclaved the solution. The media was then transferred to plates under laminar flow hood at 55°C. These plates were then inoculated with fungal strains of *A. flavus*, *A. fumigates*, *A. niger* and *F. solani* and then incubated at 27°C for growth. The solution of the plants extracts was prepared by dissolving the crude and subsequent solvent extracts in DMSO at 4mg/mL concentration. Then 1ml of each test solution was added to the fungal cultured plates. These plates were then incubated for 5to 8 days at 27°C. The inhibition of fungal growth was measured in mm (Ramirez et al., 2006; Sales et al., 2016).

RESULTS AND DISCUSSION

Presently due to the development of resistance to the available antibiotics, the hopes for the cure are again shifting towards the natural sources of remedies. The obvious choice in the situation is the plants source. Plant kingdom has been traced back to prehistorical times as a remedy for different diseases. They may be used in raw form or as decoction; some of the plants and herbs are in use as food ingredient without knowing their medicinal importance. In this connection we selected three plants which are an important part of our daily usage, but been very little explored for their effectiveness against some common pathogens. The previous reports show the presence of terpenes, flovones, alkaloids, tannins, saponins and glycosides (Rajesh et al., 2013; Sawant and Godghate, 2013; Mokhber-Dezfuli et al., 2014; Ahmed et al., 2017; Sequeda-Castañeda et al., 2019). In the current study, the antibacterial activities of the *F. vulgare,C*. *longa* and *B. vulgaris* were studied against four bacterial strains, that is, *E. coli, S. typhi*, *M. gallisepticum* and *S. dysenteriae.* These results are given in Tables 1 and 2 and Figures 1 to 6. Results in Table 1 depict that crude and *n*-hexane fractions of *F. vulgare* were effective against all selected bacterial strains. Excellent activity was shown by *n*-hexane fraction against *S. dysenteriae*, 28 mm followed by chloroform fraction 26.5mm of the same plant. Ethyl acetate fraction also showed promising activity against *S. dysenteriae* 24 mm but it was inactive against *M. gallisepticum*. The aqueous fraction of *F. volgare* was completely inactive against *E. coli*, *S. typhi* and *M. gallisepticum* except *S. dysenteriae* which was noted to be 4 mm. The activity may be due to the presence of less polar terpenes and flavones. The antibacterial activities of various fractions of *C. longa* are also appended in Table 1. From the table it is clear that the methanol and *n*-hexane fractions of *C. longa* showed promising activity against all the tested bacterial strains. Methanol fraction exhibited best activity of 23 mm against *E. coli* and least activity 15 mm against *M. gallisepticum*. The most prominent activity of 30 mm was shown by *n*-hexane fraction of *C. longa* while it was least significant against *M. gallisepticum* which was measured to be 11 mm. The recorded activity of chloroform was 16 mm against *E. coli* and 14.5 mm in *M. gallisepticum*. This fraction was completely inactive against *S. typhi.* Ethyl acetate fraction was most effective against *S. dysenteriae* measured to have 28.5 mm zone of inhibition. These activities might be due to the presence of alkaloids or some saponins present in the methanolic extract. The aqueous fractions of *F. vulgare* and *C. longa* showed almost similar zone of inhibition 0-4 mm against all the tested pathogens.

Mohsenzadeh (2007) reported that the essential oil extracted from the fruits of *F. vulgare* exhibited antibacterial activity against food borne pathogens such *as E. coli, B. megaterium* and *S. aureus*. Kaur and Arora (2008) reported that aqueous and organic extracts of *F. vulgare* showed antibacterial activity against some bacterial strains. Alcoholic and aqueous extracts of *F. vulgare* are active against *C. jejuni* and *H. pylori* (Mahady et al., 2005). The antifungal activity is reported for the fennel essential oil while its essential oil and crude seed extract is reported to posse's activity against mycobacteria and candida (Abed, 2007). Similarly the crude bark extracts of *F. vulgare* has also shown activity against Candida (Pai et al., 2010). The essential oil of *F. vulgare* has been reported to show maximum activity against *A. niger, A. flvus* and *Fusarium moniliforme* at 6 µL dose (Singh et al., 2006). The crude bark extracts of *F. vulgare* have also been reported to possess antifungal activity against *C. albicans* (Pai et al., 2010).

Results in Table 1 shows that the chloroform and Ethyl acetate fractions of *B. vulgaris* have good activity against *E. coli*, *S. typhi* and *M. gallisepticum*. The *n*-hexane fraction was also effective against *E. coli* and *M. gallisepticum* but inactive against for *S. typhi*, while the methanol fraction of the same plant was completely inactive against all the tested bacterial strains. Freile et al. (2003) reported the antimicrobial effect of *Berberis* aqueous extracts against different *Candida* species through MIC from 16 µg/ml (*Candida krusei*) to >128 µg/ml (*Candida haemulonii*). Similarly it is reported that methanolic extract of Berberis show good activity against *C. albicans, C. krusei* and *C. tropicalis* but not against *C. parapsilosis* (Iauk et al., 2007).

Niamsa and Sittiwet (2009) reported the antibacterial study on aqueous extract of *C. longa* rhizome against *S. epidermis* ATCC 12228, *Staph. aureus* ATCC 25923, *K. pneumoniae* ATCC 10031, and *E. coli* ATCC 25922.O.A. Lawhavinit et al. (2010) reported that the hexane and methanol extracts of *C. longa* demonstrated antibacterial effect against 13 bacteria.

The results in Table 2 shows the antifungal activities of

Table 1. Antibacterial activities of various medicinal plants 3 mg/mL of extracts.

Plant sample	Extract (mg/µL)	Zone of inhibition (mm)			
		E. coli	***S. typhi***	***M. gallisepticum***	***S. dysenteriae***
F. vulgare	Crude	08	11.5	17	13
	n-Hexane	20	17	26	28
	Chloroform	12	26.5	0	0
	Et.acetate	4	14	0	24
	Aqueous	0	0	0	4
	Standard	28**	30**	32*	30**
C. longa	Crude	23	21	15	20
	n-Hexane	13.5	30	11	15
	Chloroform	16	0	14.5	04
	Et.Acetate	18	10	0	28.5
	Aqueous	04	0	0	02
	Standard	28**	30**	32*	30**
B. vulgaris	Crude	05	05	05	05
	n-Hexane	9.5	0	17.5	0
	Chloroform	18	14	17	0
	Et. Acetate	15	20	28	0
	Aqueous	03	0	02	0
	Standard	28**	30**	32*	30**

(0), No inhibition zone; **ciprofloxacin (**),**30 µg/6 µl; **azithromycin (*),** 50 µg/6 µl.

Table 2. Antifungal activities of various medicinal plants 4 mg/mL of extracts.

Plant sample	Fractions	***A. flavus***	***A. fumigatus***	***A. niger***	***F. solani***
F. vulgare	Crude	12	05	7	0
	n-hexane	10	13	11	09
	Chloroform	4	0	0	04
	Et.Acetate	15	8	10	14
	Aqueous	3	2	0	0
	Standard	25***	26***	25***	30***
C. longa	Crude	15	12	12	11
	n-hexane	0	0	0	10
	Chloroform	0	19	10	2
	Et.acetate	09	22	21	08
	Aqueous	0	0	0	04
	Standard	25***	26***	25***	30***
B. vulgaris	Crude	16	15	10	0
	n-hexane	12	0	8	12
	Chloroform	11	10	3	18
	Et.acetate	0	0	10	5
	Aqueous	2	0	4	1
	Standard	25***	26***	25***	30***

(0), No inhibition zone; clotrimazole (***), 50 µg/6 µl.

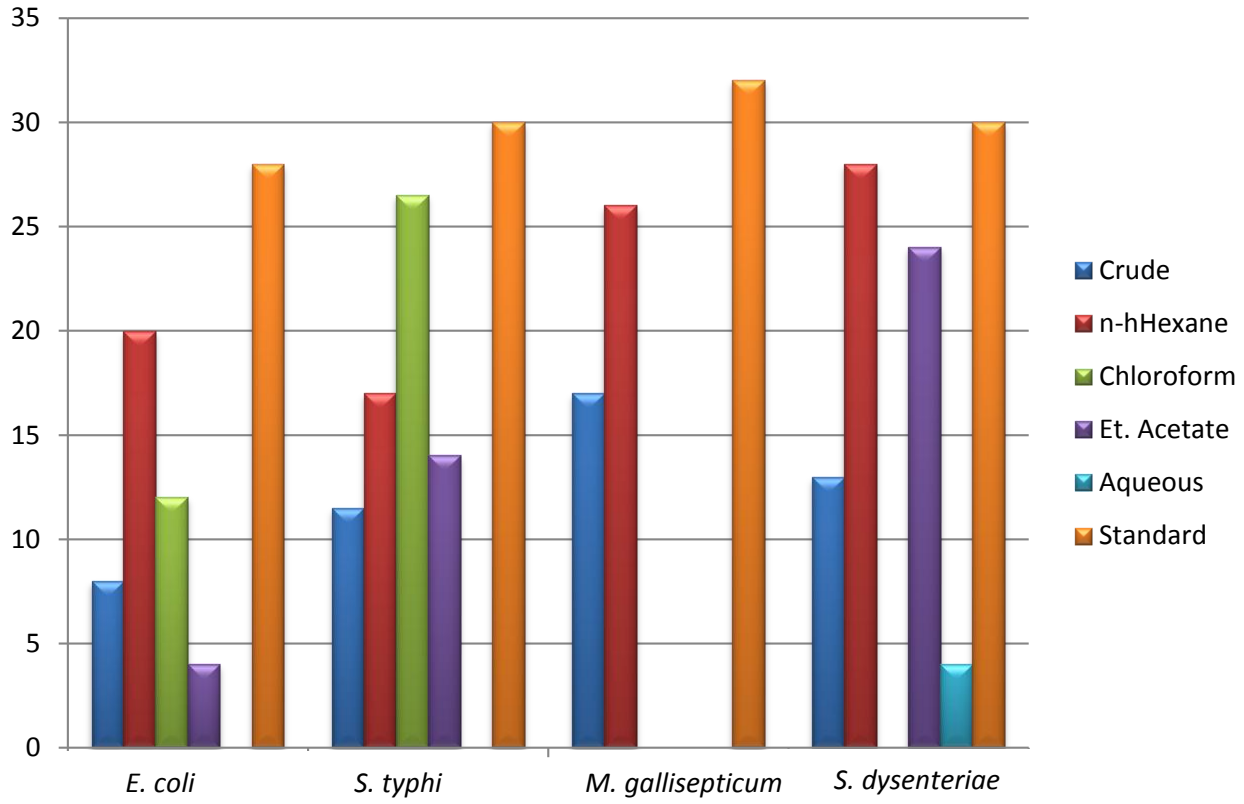

Figure 1. Antibacterial activities of *F. vulgare* 3 mg/mL.

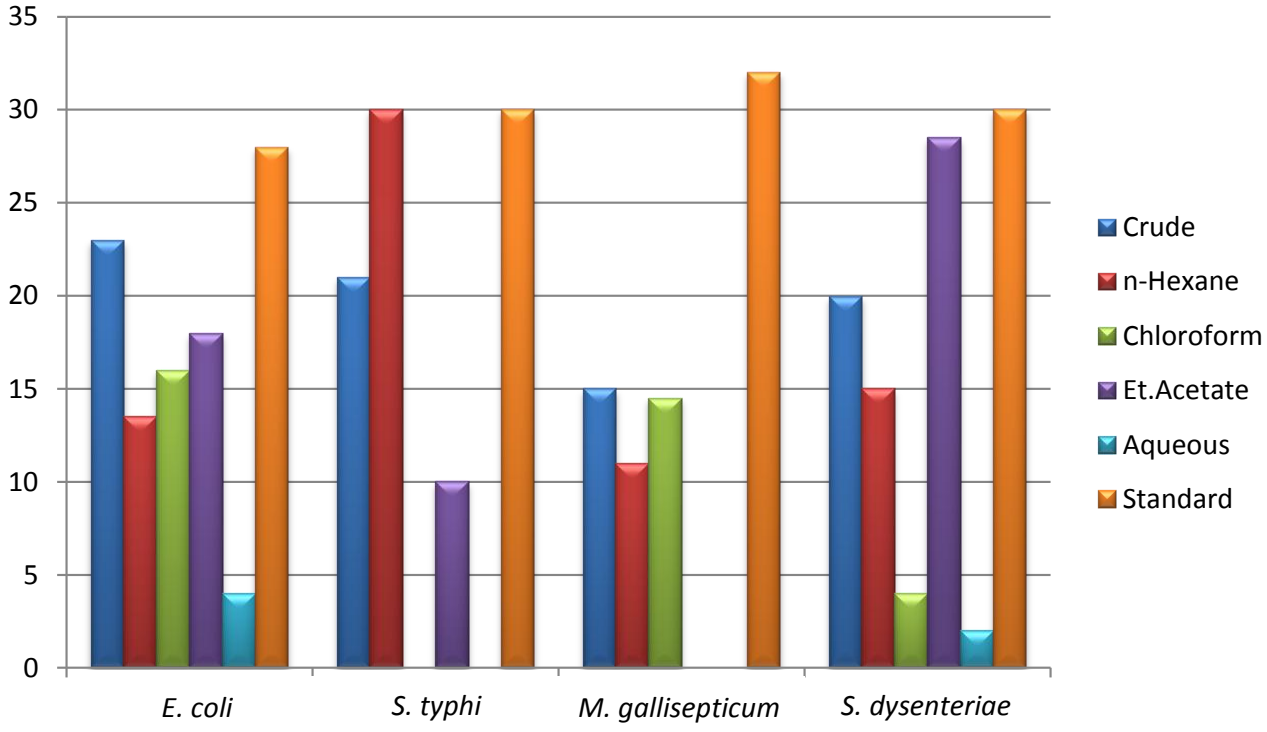

Figure 2. Antibacterial activities of *C. longa* 3 mg/mL.

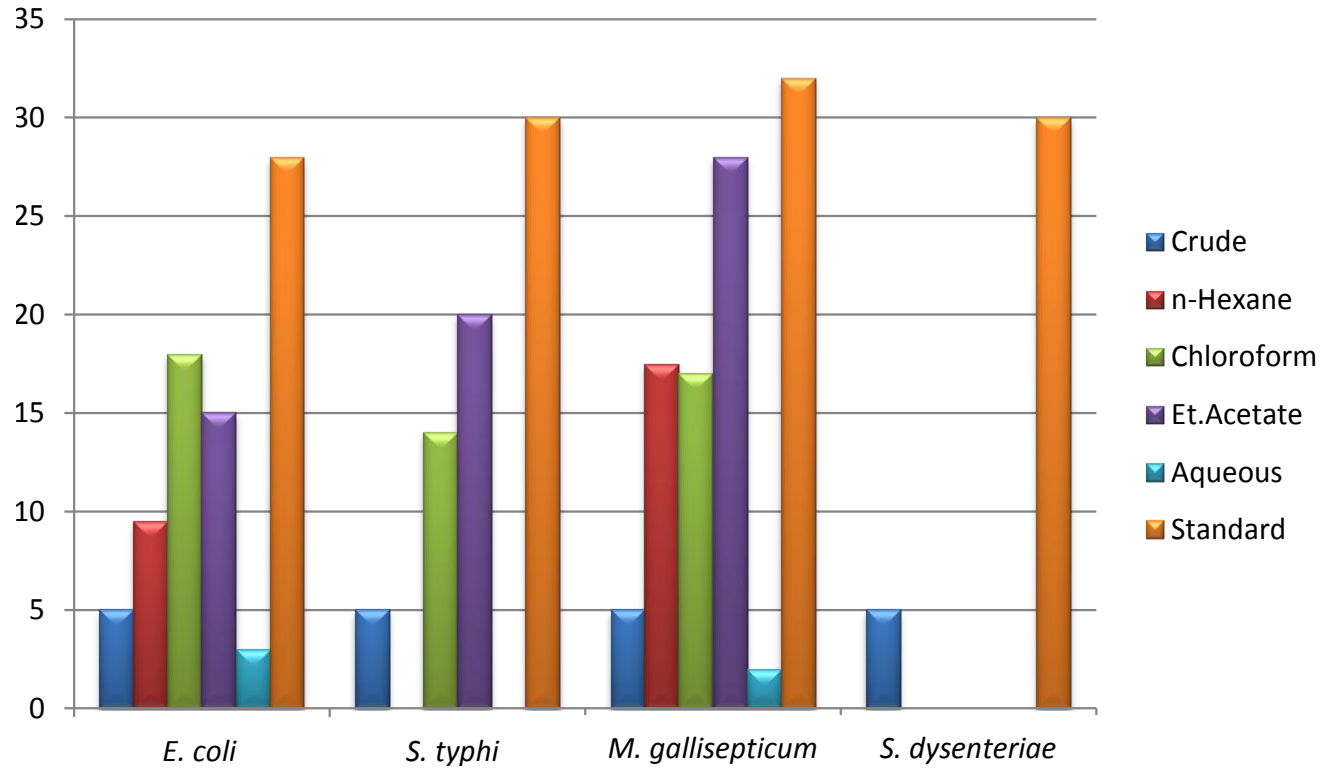

Figure 3. Antibacterial activities of *B. vulgaris* 3 mg/mL.

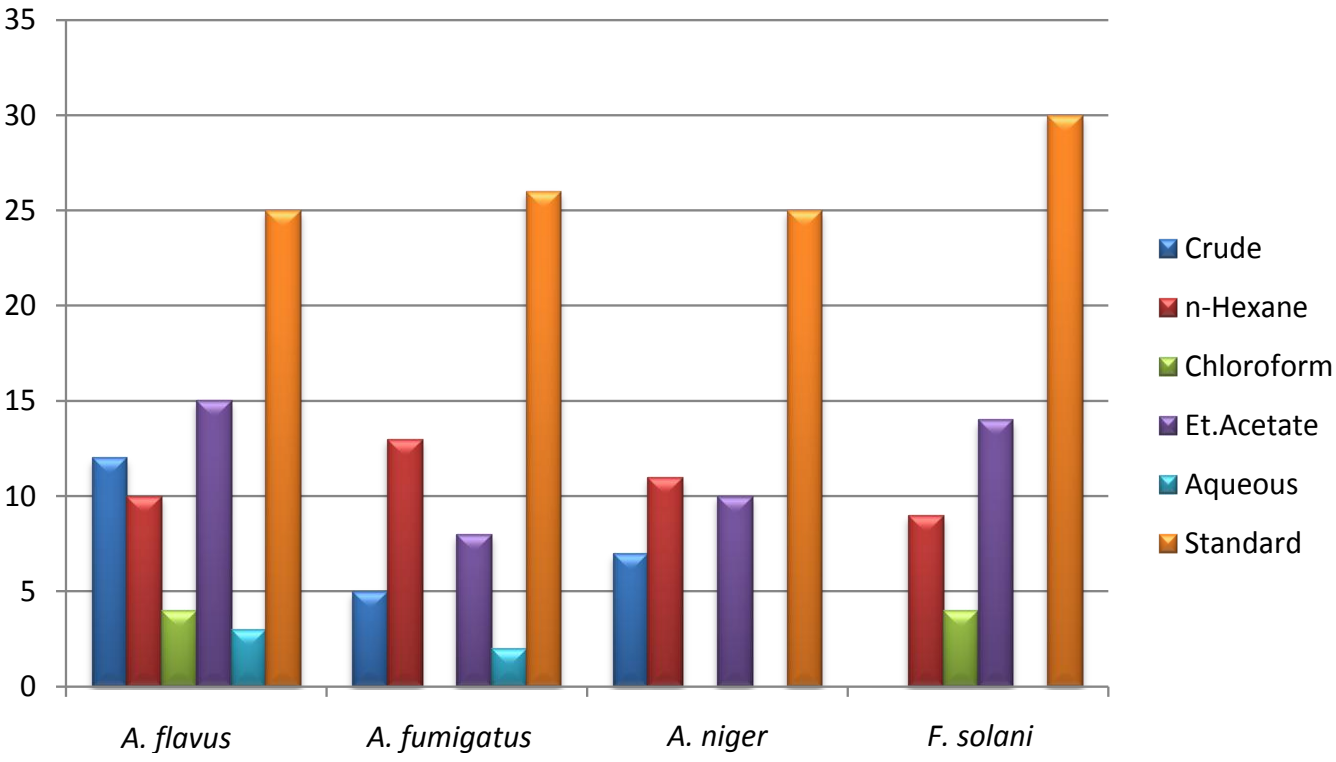

Figure 4. Antifungal activities of *F. vulgare* 4 mg/mL.

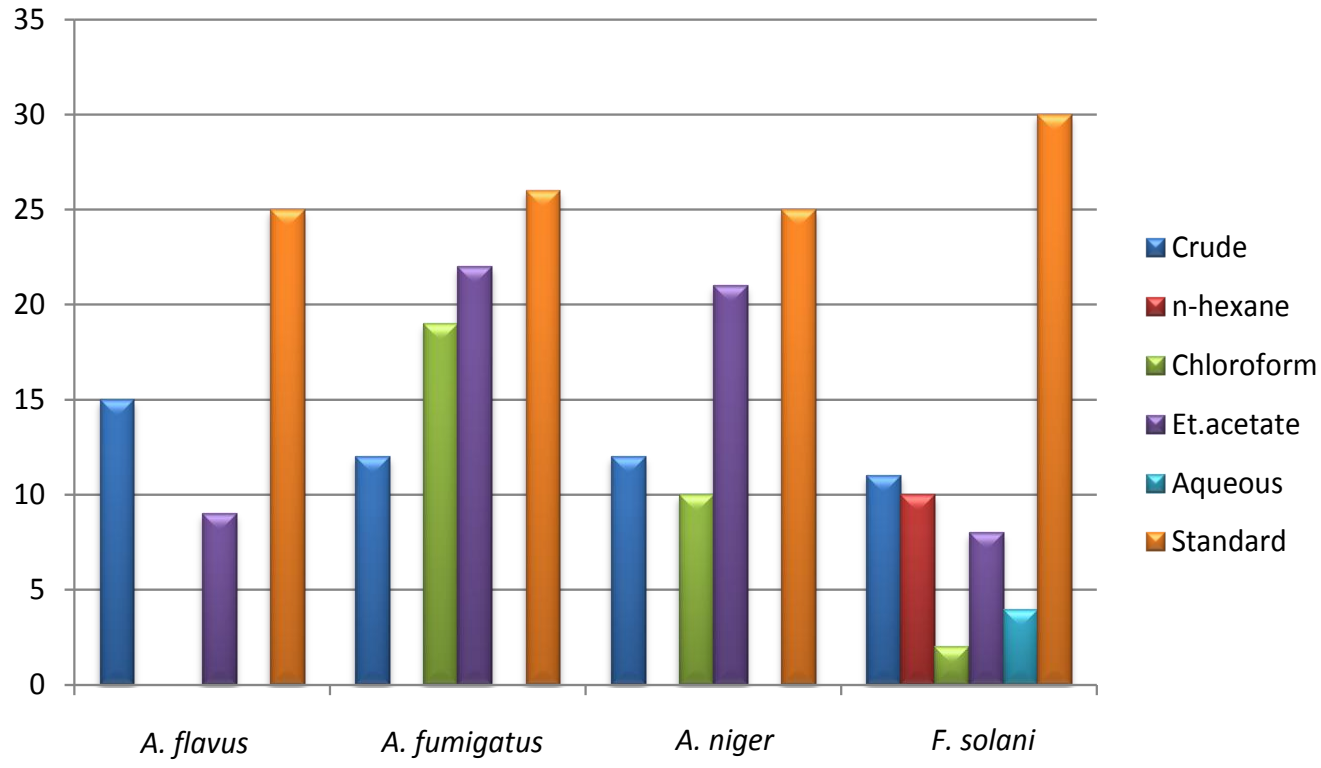

Figure 5. Antifungal activities of *C. longa* 4 mg/mL.

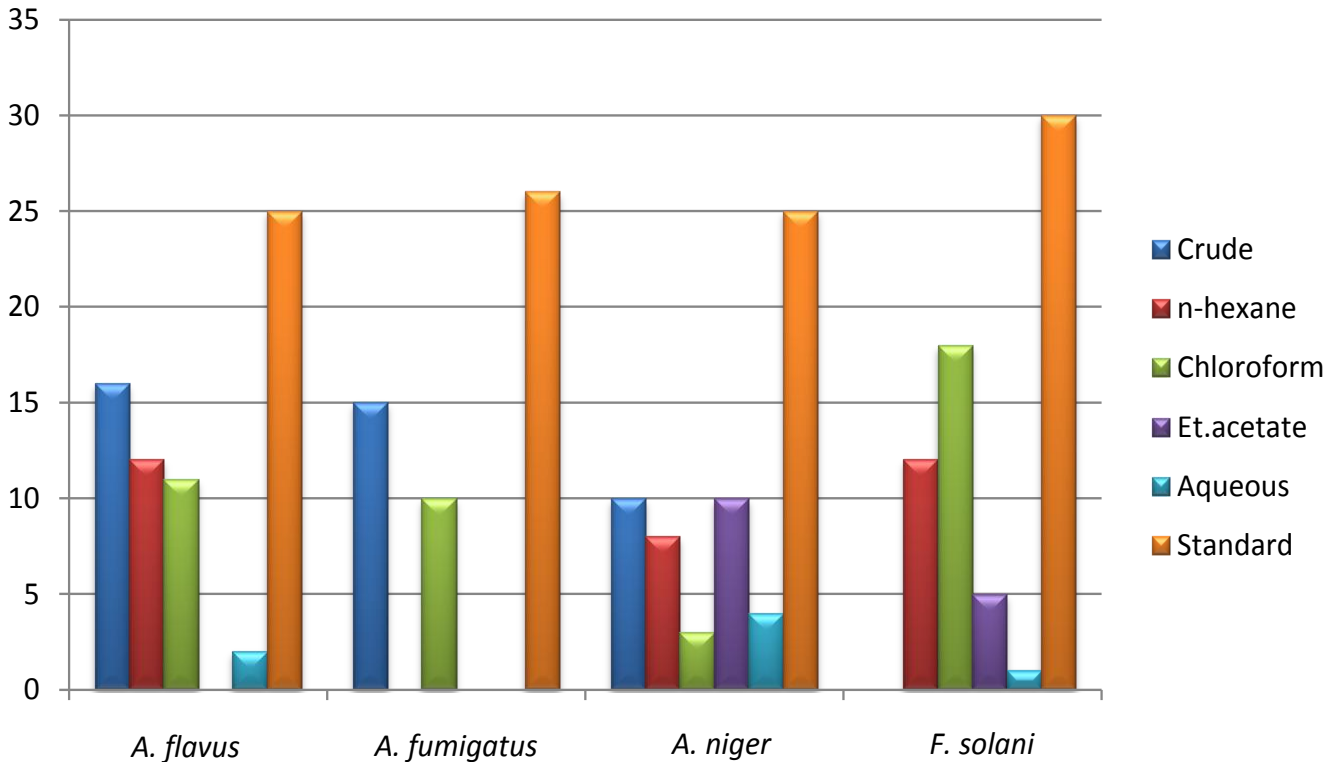

Figure 6. Antifungal activities of *B. vulgaris* 4 mg/mL.

three plants namely *F. volgare*, *C. longa* and *B. vulgaris* against four fungal strains that is, *A. flavus*, *A. fumigates*, *A. niger* and *F. solani.* The results show that *n*-hexane and ethyl acetate fractions of *F. vulgare* were more active against the tested fungal strains as compared to methanol and chloroform fractions. The highest activities were shown by ethyl acetate against *A. flavus* 15 mm and *F. solani* 14 mm. The chloroform fraction of the same plant was completely inactive against *A. fumigates* and *A. niger*. Similarly the activity of aqueous fraction was not significant against the tested fungal strains. The methanol and ethyl acetate fractions of *C. longa* were active against all the tested fungal strains but the activities of n-hexane and water fractions were not significant. Whereas measured zone of inhibition of chloroform against *A. fumigates* was 19 mm. The highest zone of inhibition of ethyl acetate fraction, 22 mm was measured against *A. fumigates*. The *n*-hexane fraction of the same plant was entirely inactive against *A. flavus*, *A. fumigates* and *A. niger* except *F. solani* for which inhibition zone of 10mm was recorded. Similarly the activities for various fractions of *B. vulgaris* were presented in Table 2 and are graphically represented in Figures 4, 5 and 6 from which shows that all the fractions were active against the *A. niger*. Methanol fraction was significant against all the tested strain except *F. solani*. The most promising activities were shown by crude fraction against *A. flavus* 16mm and chloroform fraction against *F. solani* 18 mm.These activities might be due to the presence of the less polar alkaloids in the fractions.

Conclusion

The present investigations suggest that most fractions of *F. vulgare*, *C. longa* and *B. vulgaris* have good antimicrobial properties that can be preceded further for new source of antibiotic drugs and can also be used for the control and treatment of infection. Due to presence of good microbial agents in these plants, further studies are required to isolate and characterize the active component of the extract and also to elucidate their antimicrobial mechanism of action.

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