Original Article

ANTIBIOGRAM OF *TRACHYSPERMUM AMMI* (AJWAIN) AGAINST TEST MICROORGANISMS

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Abstract:

Statement of problem In the existing scenario, where the continuous rise in emergence of multi resistance pathogens is posing serious health threats, there is an urgent need to find out other (preferably natural) sources for safe and effective therapeutic agents as alternative to antibiotics.

Objectives: The main objective is to find out and evaluate the antimicrobial potential of trachyspermun ammi against two most problematic pathogens (gram +ve *S.aureous* and gram –ve *S.typhi*)

Study design: Experimental / in vitro study conducted in department of School of pharmacy, The University of-Faisalabad during September 2015 to February 2016.

Methodology: Antimicrobial activity of crude extract of *trachyspermun ammi* was evaluated by standard Disc Diffusion Method and compered with standard antibiotic (ciprofloxacin).

Results: Crude extract of *trachyspermun ammi* manifested remarkable antibiotic activity (30.33±0.33mm) against test organisms even surpassing the activity of control antibiotic (29.17±0.17mm).

Statistical analysis: Investigated plant extract revealed significant antibacterial activity, which was significantly more /greater than the reference drug.

Conclusion: Study finding indicate the presence of highly potent broad spectrum antimicrobial compounds/agents in the plant under investigation (*trachyspermun ammi*).

Key Words: *Trachyspermun Ammi*, Antimicrobial activity, problematic pathogens, Disc Diffusion method, Ciprofloxacin, S. typhi, S. aureus

BACKGROUND:

Currently the society is facing one of the most challenging public health dilemmas, because of threatening problem of substantial and continuous rise in emergence of multi drug resistance microbes worldwide[1,2,3.] According to WHO, multi drug resistance to conventionally used antibiotics were observed to be 65% in Pakistan 20% in Viet Nam, and 7% in India [4].

Researchers all over the world are focusing on medicinal plants for alternative source for safe and effective antimicrobial therapeutic agents. Spices are important medicinal plants that has been used in traditional medicines.

Ajwain is counted among the most used spices in various parts of the world, and is used for numerous culinary purposes. Fruits/seeds have long been highly administered by traditional healers worldwide, and traditionally implied for different ailments. With reference to its pharmacological

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activities, the traditional knowledge still remained to be confirmed scientifically. This important postpartum herbal care traditional medicine is being actively investigated for its pharmacological activities, and the results are remarkable. This may contribute to new plant based effective latest antibiotic formulation [5].

INTRODUCTION:

Infectious diseases are the leading cause of death worldwide [6]. Excessive use of antibiotics may result in serious changes in gut micro biota [7]. Over the last decade, some of the microorganisms have emerged as the most problematic nosocomial pathogens, multi drug resistance clones of which have been disseminative worldwide [8].

It is estimated that about 70% of bacteria that caused ailments in hospitals, become insensitive to at least one of the drugs currently prescribed for treatment[9]. Continuous spread of multi drug resistance pathogens, increasing cost of drug regimens and increasing side effects of current allopathic medicine, the scenario has paved the way for new and reemerging infectious diseases worldwide [10].

So, to address the ever increasing resistance of pathogens, cost of antimicrobial agents and their side effects, there is an urgent need to put attentions to natural sources (medicine plant, fruits and spices etc.), for cost effective safe and natural therapeutic agents as alternative to antibiotics /modern medicines [11]. Medicinal herbs have long been believed as a source of therapeutic remedy based on sacred and cultural customs. Ajwain is one among these ancient time companion of man. Use of herbal medicine has amplified dramatically for various disease because natural remedies are supposed to have much less toxic effects as compared to synthetic medicine [12].

***TRACHYSPERMUM AMMI* (AJWAIN):**

PLANT DESCRIPTION:

This is a glabrous annual herbaceous plant (60—90cm tall) belonging to highly valued and medicinally important family Apiaceae or umbellifera. Its stems are hallow, striate and profusely branched, and grayish brown aromatic fruits/seeds; popular and highly valued seed spice that has been extensively used for treating number of diseases/disorders in human, that are considered to be the most useful parts of the herb due to their nutritional and medicinal values [5].

PHOTOCHEMISTRY:

The alcoholic extract has highly hygroscopic seponin, crystalline flavone and a steroidal substance. The principle constituents of essential oil (2-4%) from seeds are the phenols mainly thymol (35-60% and some carvacol). Fixed oils extracted contains resin acids, palmitic acid, petroselenic acid, oleic acid and linoleic acid.

Seed analysis revealed fiber 11.9%, carbohydrate 38.6%, tannins, glycosides, moisture 8.9%, protein 15.4%, fat 18.1%, mineral matter 7.1% containing calcium, phosphorus, iron and nicotinic acid [13,5].

Uses: Parts of the plant used are; whole plant, fruits/seeds and oil.

Culinary use: Spice is used for numerous culinary purposes [13].

Medicinal use: In Ayurveda, Unani, Medieval and Traditional Persian Medicine, this is extensively used for different disorders of respiratory, digestive and urogenital system and also for skin disorders [5].

Economic use: Seed extracts are added to cough medicines, soaps and epoxy derivative, mouth washes, tooth paste, perfumery and various cosmetics [13].

Ornamental use: Plant is used for ornamental purpose [13].

Current Pharmacological findings/studies: Currently pharmacological effects are being actively investigated.It has antibacterial and antifungal[14], Anti-helmintic [15], effect on intestinal flora [16], Antiulcer [17], Antidiuretic [18], Detoxification [19], Ameliorative [20], Antioxidant [21], Blood coagulation [22] and Analgesic and Anti-nociceptive effects [23].

Traditional Recommended dosage range is; [24].

Seeds - 1 to 3 grams.
Oil – 1 to 3 drops.
Distilled – 5 to 10 drops.

Test Organisms:

Most common problematic pathogens, one from gram positive and other from gram negative bacteria, are selected as test organisms.

Salmonella typhi: Gram negative bacterium responsible for typhoid global burden/typhoid fever; which is still on rise, especially in high risk areas of the world [25, 26].

***Staphylococcus aurous*:** Gram positive bacterium is major pathogen for human that is rapidly acquiring multi drug resistance and can cause suppuration in any organ/system [27].

OBJECTIVES:

1. To investigate the anti-microbial (gram negative and gram positive) potential of *Trachyspermum ammi*.
2. To provide preliminary information(s) for further development of more potent broad spectrum antibiotics effective against multi resistant pathogens, particularly against *Salmonella* (responsible for deadly typhoid fever), which is sensitive only to very few standard antibiotics presently.

MATERIAL AND METHODS:

Methodology:

Antibiotic assay is performed by using the disc diffusion methods.

Extract Preparation:

The spices was grinded to make fine powder. Fine powder of spice, *Trachyspermum ammi*, was taken. 30gram of spice was mixed with 100ml of ethanol and 100ml of water separately. Extracts were prepared with the help of Soxhlet apparatus as well as simple maceration process. Then 10µl of each extract was used for anti-bio gram.

Table: 4.1 Botanical information of the spice used

Sr. #	Botanical Name of Plant	Common Name (English)	Common Name (Urdu)	Family	Part Used.
1.	*Trachyspermum ammi*	Ajowan	Ajwain	Apiaceae	Seeds

Fig: 4.1 *Trachyspermum ammi* (Ajowan) Plant Fig: 4.2 *Trachyspermum ammi* (Ajwain) Seeds

Inoculum Preparation:

The test organism of each strain was sub cultured on nutrient agar medium (by incubating at 37 degree overnight), and from this fresh culture, standard size (10^8to10^9 CFU per ml) inoculum is prepared in used.

Preparation of Disc:

What Mann No. 1.6mm filtered paper antibiotic (extract) discs were prepared and sterilized by autoclaving.

Inoculum and Testing:

Antimicrobial activity of the extracts was tested using the disc diffusion method 10µl of each extracts was impregnated into empty sterilized antibiotic disc. Each Muller Hinton agar plate was inoculated with the standard inoculum suspension by soaking a swab and rotating it over the agar plate. The paper antibiotic discs were placed over the inoculated agar. After 24 hours of incubation at 37°c, zones of an inhibition were measured and recorded.

Reference drugs: Followings are used as reference:

- Ciprofloxacin 5µg of composed disc was used as control positive.
- Ethanol was used as negative control.
- Distilled water was used as negative control.

Antimicrobial Bioassay:

Antimicrobial activities of different extracts were determined by Agar Diffusion method [28]. For this, nutrient agar was used as culture media, Cotton swabs were dipped in the standard size inoculum and were swabbed on the solidified media surface. Discs were placed aseptically over the standard size inoculum on the nutrient agar plates along with positive and negative controls and incubated at 37^0c for 24 hours. All treated petri plates were immediately placed in incubator at 37^0c. Sterile, blank paper discs impregnated with only sterile water and ethanol were used as negative control each time. Standard Ciprofloxacin was used as positive control for comparison of antibacterial activity. After 24 hours incubation, all the plates having discs for alcoholic extracts and water extracts were observed for their zone of inhibition . The zone of inhibition around the discs were measured in millimeter (mm) by venire caliper. The data of zone of inhibition of spice extract, having two types of solvent extracts (water and ethanol) and a control against two bacterial pathogens was recorded with six times repeats to confirm the reproducible results of plant extracts. Since all the observations in negative control were zero, therefore data in negative control was not used for statistical analysis.

Fig 4.3 Zone of inhibition of ajwain (by water maceration)

Fig 4.4 Zone of inhibition of ajwain (by soxhlet)

Fig 4.5 Zone of inhibition of ajwain (by ethanol maceration)

Statistical Analysis:

Data was analyzed by two way analysis of variance. The data collected for each experiment are subject to statistical analysis. Data was analyzed using (SPSS) Statistical Package for the Social Sciences. The data were presented in tabulated form (Table 5.1 - 5.9) and graphical form (Fig 5.1)

RESULTS:

It was observed that:

- *Trachyspermum ammi* revealed more antimicrobial activity (30.33±0.33mm) than ciprofloxacin (29.17±0.17 mm).
- *Trachyspermum ammi* was more effective against *Salmonella typhi* (30.33±0.33mm) than *Staphylococcus aureous (*28.00±0.45mm).
- Alcoholic extracts revealed more antibacterial activity (30.33±0.33mm) than water extracts (17.50±0.34mm).
- Extract prepared by soxhlet method revealed more antimicrobial activity (30.33±0.33mm) than the extracts prepared by maceration method (28.50±0.72mm).

Table 5.1 Analysis of variance (by Soxhlet)

Source	DF	SS	MS	F	P
Organism	1	301.04	301.04	179.73**	0.000
Treatment	1	92.04	92.04	54.95**	0.000
Organism*Treatment	1	45.37	45.37	27.09**	0.000
Error	20	33.50	1.68		
Total	23	471.96			

** = Highly significant (P<0.01)

Table 5.2 Comparison of Antimicrobial activity (in millimeter) of Alcoholic crude extract (by soxhlet extraction) of Trachyspermum ammi spice with ciprofloxacin (standard drug) against test organisms

Organism	Treatment		Mean
	Plant extract	Ciprofloxacin	
S.aureus	26.00±0.97b	19.33±0.21c	22.67±1.11B
S.typhi	30.33±0.33a	29.17±0.17a	29.75±0.25A
Mean	28.17±0.81A	24.25±1.49B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5.3 Analysis of variance (Ethanolic by maceration)

Source	DF	SS	MS	F	P
Organism	1	15.042	15.042	12.62**	0.002
Treatment	1	176.042	176.042	147.73**	0.000
Organism*Treatment	1	7.042	7.042	5.91*	0.025
Error	20	23.833	1.192		
Total	23	221.958			

** = Highly significant (P<0.01)

Table 5.4Comparison of Antimicrobial activity (in millimeter) of Alcoholic crude extract (by maceration extraction) of Trachyspermum ammi spice with ciprofloxacin (standard drug) against test organisms

Organism	Treatment		Mean
	Plant extract	Ciprofloxacin	
S.aureus	28.00±0.45a	21.50±0.22c	24.75±1.01B
S.typhi	28.50±0.72a	24.17±0.17b	26.33±0.74A
Mean	28.25±0.41A	22.83±0.42B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5.5Analysis of variance (Aqueous maceration)

Source	DF	SS	MS	F	P
Organism	1	66.667	66.667	88.89**	0.000
Treatment	1	150.000	150.000	200.00**	0.000
Organism*Treatment	1	4.167	4.167	5.56*	0.029
Error	20	15.000	0.750		
Total	23	235.833			

** = Highly significant (P<0.01)

Table 5.6 Comparison of Antimicrobial activity (in millimeter) of Alcoholic crude extract (by maceration extraction) of Trachyspermum ammi spice with ciprofloxacin (standard drug) against test organisms

Organism	Treatment		Mean
	Plant extract	Ciprofloxacin	
S.aureus	13.33±0.56d	19.17±0.17b	16.25±0.92B
S.typhi	17.50±0.34c	21.67±0.21a	19.58±0.66A
Mean	15.42±0.70B	20.42±0.40A	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5.7 Analysis of variance

Source	DF	SS	MS	F	P
Organism (O)	1	288.00	288.00	238.89**	0.000
Treatment (T)	1	37.56	37.56	31.15**	0.000
Extract (E)	2	1018.69	509.35	422.50**	0.000
O x T	1	18.00	18.00	14.93**	0.000
O x E	2	94.75	47.37	39.30**	0.000
T x E	2	380.53	190.26	157.82**	0.000
O x T x E	2	38.58	19.29	16.00**	0.000
Error	60	72.33	1.21		
Total	71	1948.44			

** = Highly significant (P<0.01)

Table 5.8 Overall Comparison of Antimicrobial activity (in millimeter) of Ethanolic (by soxhlet) Ethanolic (by maceration) water extract (by maceration) of Trachyspermum ammi spice with ciprofloxacin (standard drug)

Extract	Treatment		Mean
	Plant extract	Ciprofloxacin	
Ethanolic extract (Soxhlet)	28.17±0.81a	24.25±1.49b	26.21±0.92A
Ethanolic extract (Maceration)	28.25±0.41a	22.83±0.42c	25.54±0.63B
Aqueous extract (Maceration)	15.42±0.70e	20.42±0.40d	17.92±0.65C

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table 5.9 Overall Comparison of Antimicrobial activity (in millimeter) of Ethanolic (by soxhlet) Ethanolic (by maceration) water extract (by maceration) of Trachyspermum ammi spice with ciprofloxacin (standard drug) against test organisms

Organism	Extract	Treatment		Mean
		Plant extract	Ciprofloxacin	
S. aureus	**Ethanolic (Soxhlet)**	26.00±0.97c	19.33±0.21f	22.67±1.11D
	Ethanolic (Maceration)	28.00±0.45b	21.50±0.22e	24.75±1.01C
	Aqueous (Maceration)	13.33±0.56h	19.17±0.17f	16.25±0.92F
S. typhi	**Ethanolic (Soxhlet)**	30.33±0.33a	29.17±0.17ab	29.75±0.25A
	Ethanolic (Maceration)	28.50±0.72b	24.17±0.17d	26.33±0.74B
	Aqueous (Maceration)	17.50±0.34g	21.67±0.21e	19.58±0.66E

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean.

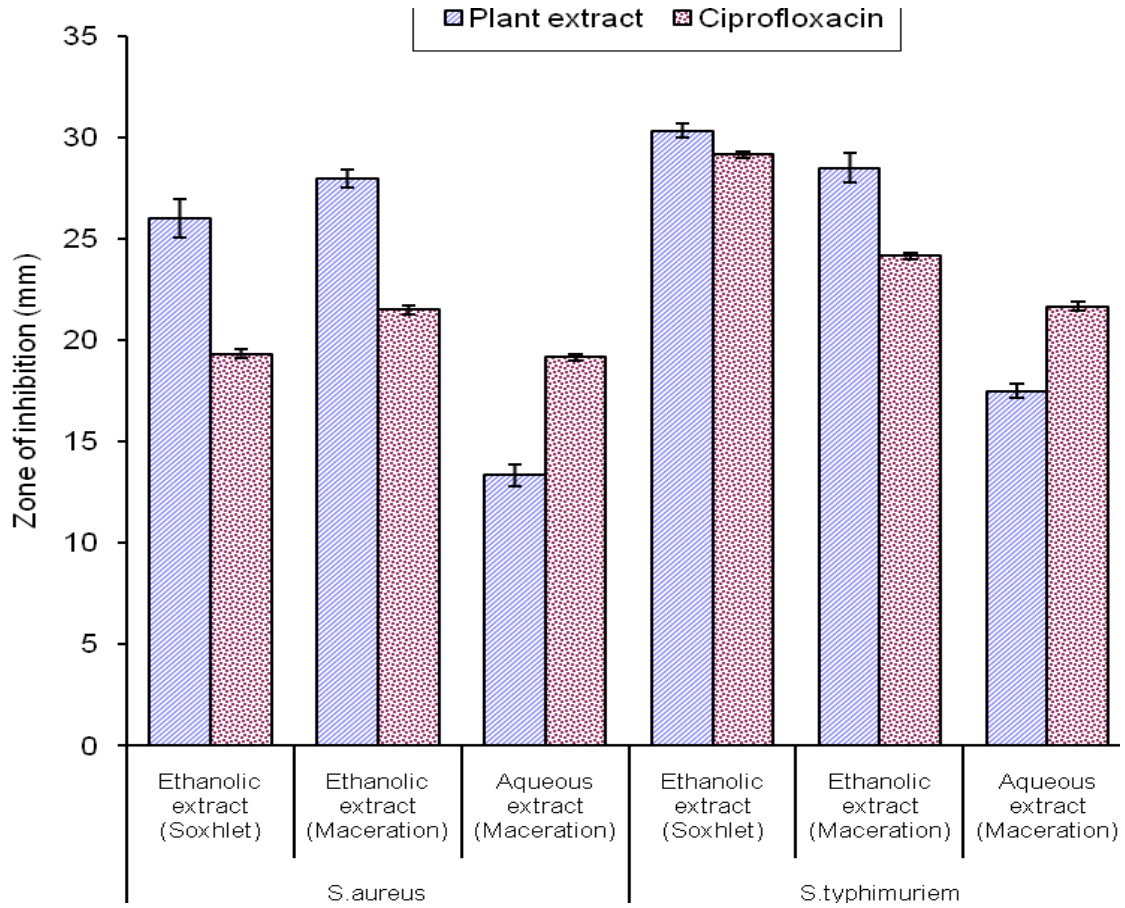

Fig 5.1: Overall Comparison of Antimicrobial activity (in millimeter) of Ethanolic (by soxhlet) Ethanolic (by maceration) water extract (by maceration) of Trachyspermum ammi spice with ciprofloxacin (standard drug) against test organisms

(i) **Ciprofloxacin** (reference drug) showed zone of inhibition ranging from19.17±0.17mm (*S.aureous*) to 29.17±0.17mm *(S.typhi).*

(ii) **Plant extracts:** All extracts manifested varying but significant degree of activity against two tested bacteria.

(iii) **Solvents:** Among the solvents ethanol showed better extraction power than distilled water.

(iv) **Methods:** Soxhlet method proved more effective.

(v) **Bacteria:** Both bacteria tested proved susceptible to the plant extracts evaluated. Among these tested organisms, *S.typhi* showed highest zone of inhibition 30.33±0.33mm.

DISCUSSION:

The important findings of present study are:

1. All spice extracts showed significant antimicrobial activity against both clinically important pathogens.

2. Magnitude of antimicrobial activity of different extracts varied significantly according to the solvent used, as well as method of extraction applied.

3. Highly significant finding was, that spice extract exhibited better antimicrobial

activity than the Ciprofloxacin (positive control).

***Trachyspermum ammi* (Ajwain):**

It showed marked inhibition for both G negative and G positive bacteria even comparable to reference drug. It not only inhibit *S.typhi*, but also showed remarkable antimicrobial activity against *S.aureus*. So, it can nicely contribute to effective plant based antimicrobial formulation against multi drug resistant (MDR) microbial pathogens, and also against *S.typhi* (responsible for life threatening typhoid fever), for which we have very limited number of effective antibiotics.

Ajwain, a highly valued medicinally important seed spice that has widely been employed for treating number of diseases in human. In Indian system of medicine it has been used for curing typhoid fever. Phenolic compounds (carvacol and thymol) considered to be the active principles, displaying strong antimicrobial potential against the microbes resistant to even the latest generation of antibiotics [20].

Ajwain is a one of the most important herbs that are used in post-partum care and food supplement in Subcontinent. These are scientifically proved to be responsible for significant anti-oxidant, anti-inflammatory and antimicrobial activities [29].

Recently, this medicine plant is proved to be a good R plasmid curing agent [30], so, it can play vital role in plant based herbal antibiotic formulation against multi drug resistant (MDR) microbial pathogens.

However, still more research work is required to determine the pharmacokinetics, biochemical, pharmacodynamics and therapeutics of active components and their actions with modern drugs and importance to human health with sufficient scientific data.

METHODS:

Soxhlet method proved to be better because different conditioning factors (temperature, pressure and pH etc.) can properly/effectively be controlled by this apparatus.

Solvents:

In present study, the ethanol proved to be better extractor, for this selected plant, as were the cases of previous studies in number of other different plants [31, 32, 33, 34].

But in other studies, different extracting solvents proved to be more effective for different plants. Methanol proved to be a better extractor for *psidium guajava, nigella sativa, citrus sinensis, valerian jutamansi* and *cucurbita papa* [35].

Other extractors; Chloroform for *Foenicolum valgare Mill* [36], distilled water for *Lawsonia innernis Linn* [37], and petroleum ether for *Trigonella foenum- graecum* [38] proved more effective than other solvents.

Differential extraction ability/power of solvents may be because of different solubility of different active principles of different plants in different solvents. So, it appears to be related with differential solubility of active principles of plants for different solvents. It is thus concluded that in preparation of extracts, the solvents used are very important factor. So, different solvents should be probed to find out the best solvent for particular plant.

Plants:

Generally a plant is consider to be active/effective against pathogenic microbes when the zone of inhibition is greater than 6mm [39]. Results showed/indicated that all spice extracts tested, significantly inhibited the growth of two tested pathogen bacteria at varying degrees. The maximum zone of inhibition 30.33±0.33mm was found with ethanol extract of spice (against *S.typhi*) and the minimum zone of inhibition 13.33±0.56mm was found with aqueous extract of spice (against *S.aureous*).

Results obtain from in vitro antimicrobial activity showed that all plant extracts exhibited a substantial/significant inhibitory effects against two tested bacteria. Ethanol extracts of plant extract from soxhlet method proved superior in suppressing the bacterial growth. This was followed by water extracts. Ethanol extracts exhibited promising antibacterial activity against two tested bacteria (both gram negative and positive), may be due to presence of phenols and flavonoids (active principles/ingredients) which are better extracted with ethanol [40]. The lesser activity seen with other solvent

(water) may be due to low solubility of active constituents/principles in this solvent [40].
Varying degrees of antimicrobial activities may be due to different;

(i) active principle(s) within the different plant (spice) extracts
(ii) solubility of active principles in different solvents
(iii) conditioning factors (temperature, pressure and pH etc.) during processing

In present study, all plant extracts revealed significant antimicrobial activity against both Gram negative and Gram positive microbes, proving their broad spectrum potential. Studies all over the world are acknowledging the different properties/activities of medicinal plants, and it seems that plants have an enormous but as yet untapped potential to yield novel drugs with desired safety profile.

Bacteria:

Regarding the susceptibility; *S.typhi* proved to be most sensitive organism to reference drug and also against the plant extract compared to the *Staphylococcus aurous.*

ACADEMIC VALUE OF STUDY:

This study provides;

i. Scientific prove/base to traditional healers claim.
ii. Bases for a full scale investigation of the therapeutic potential of this medicinal plant.

This may help to established parameters for further development of more effective broad spectrum antimicrobial (against gram negative as well as gram positive) agents, more potent antimicrobial agent against multi drug resistance strains of microbes and other pathogens with higher resistance, and also more effective and safe anti-microbial for deadly typhoid fever, against which limited anti-biotic are available at present.

LIMITATIONS OF STUDY:

Study was confined only to two bacteria. If the sensitivity of more bacteria would have been assessed, the impact of study would have carried more weightage.

CONCLsUSION:

Crude extracts of plant under investigation, significantly inhibited common medically important isolates (both Gram positive & Gram negative), proving that this plant has potential as an alternate source of broad spectrum antimicrobial agents affective against multi drug resistant (MDR) pathogens including life-threatening *S.typhi,* responsible for typhoid burden globally.

Future Recommendations:

There is need to isolate the bioactive components (bio-principles) that are responsible for the ethno-pharmacological properties of this medicinal plant. This is might be accomplished after proper purification, quality chemotherapeutic index and pharmaceutical analysis of this plant extract.

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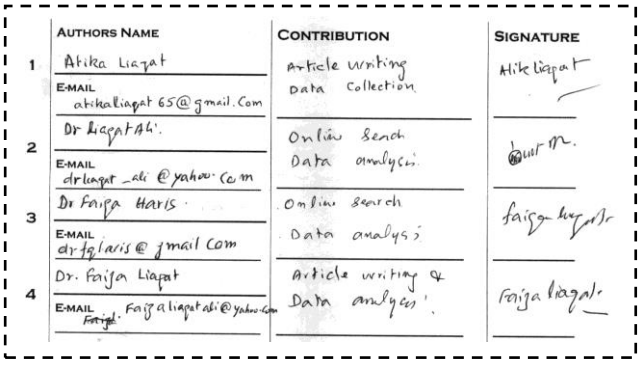

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