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Biological activities of some selected medicinal plants of Agra valley district Malakand, Pakistan

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Diseases are going to more complexity to cure by using of synthetic antibiotics. Because pathogens developing resistance against antibiotics. Plants extracts are the alternate source of providing remedies for different types of diseases. Wild plant species were collected from sub-alpine regions of Agra valley, Distract Malakand. The area is floristically rich that's why four plant species i.e *Lespedeza cuneata*, *Ficus sarmentosa*, *Heteropappus altaicus* and *Artemisia scoparia* were selected and the methanol extracts of these plants were tested for their biological activities, evaluated through Disc Diffusion Susceptibility Method. The activities of plant extracts were compared with those of standard antibiotic. The methanol extract were tested against 8 pathogenic species of microbes. The seven Bacterial species were *Bacillus atrophaeus*, *Bacillus subtilis*, *Escherichia coli*, *Citrobacter species*, *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella typhi*, while 1 species of fungus was *Candida albicans*. Their antibiotic activities were evaluated by measuring their zones of inhibition, inhibited by methanol extracts of selected plants. The major zone of inhibition was recorded for *Ficus sarmentosa* followed by *Heteropappus altaicus*. The minor activities were showed by *Artemisia scoparia* while *Lespedeza cuneata* exposed no such type of activities. It was concluded from the present study, that some plants extract having great potential against different types of pathogens. These plants are can take the alternate position of synthetic antibiotics.

Keywords: Identification of Plants, Biological activities, Agra valley. Malakand, Pakistan

INTRODUCTION

District Malakand is one of the 26 districts of Khyber Pakhtunkhwa in Pakistan. Malakand is located at 34.57, 71.93 (Lat / Lng.). The estimated total area of District Malakand is about 952 km^2 and has a population density of 596 persons per km^2 (DCR, 2017). Agra is located west side to Malakand pass. Full hills covered area, with a diverse communities of plants. The elevation of Agra valley from sea level is about 1200 meters to 2000 meters (Murad *et al*., 2011). The modern world mostly depends on the plant extracts and some other derivatives, which may be nutritional, medicinal or can also be used in cosmetics

(Alyaiev, 2007). Different Research work of on ethnobotany shows that plants are the main resource of the cure of human diseases since the beginning of human life (Bourdy *et al*., 2008; Khalil *et al*., 2014). Different traditional medical practices have been installed in various cultures that pertain to the use of different herbs and their remedies measures against different diseases (Hazrat *et al*., 210, Khalil *et al*., 2014). WHO stated that about 80 percent of the people execute their healthiness necessities from plants having some medicinal value while out of two hundred and fifty thousand to five hundred thousand plants, many plants needs to explore for the conventional use (Mahesh & Satish, 2008). In recent times the universal market of herbal and scented plants has extensively improved and is predictable to reach 5 trillion dollars by 2050 (Shinwari, 2010). According to a general estimate, there are about 35000-75000 remedial plants that are able to make a considerable involvement to accomplish the health problems (Khalil *et al*., 2013). The local inhabitants acquire abundant native information that should be acknowledged as well as significantly examined using systematic values for their confirmation (Shinwari *et al*., 2013).

Due to the overconsumption of antibiotics, the human being is at present facing a problem of a microbial population becoming defiant to the present treatment and speedily sprouting in opposition to the presently used antibiotics. One of the main complexities in the general approval to the herbal therapy is the incorporation of the conventional awareness in the modern daily remedial practices, which is hard because very slight work has been made on the substantiation of the local knowledge. It would be the first duty to file and brought conventional information to modern time systematic principles (Hazrat *et al*., 2013, Khalil *et al*., 2014).

Search for herbal medicine has sped up in current years. Pharmacists, microbiologists, biochemists botanists, and natural-products chemists throughout the world are at this time evaluating medicinal plant species for Phytochemical and lead compounds that could be used for the cure of a variety of diseases (Acharya *et al*., 2008). As stated by the world Health Organization about 60 percent of the world's population depends on conventional medicine, and 80% of the population in developing countries rely almost entirely on conventional medical practices, mainly used particularly, herbal medicine for their primary health care needs. (Famsworth *et al*., 1994 and Zhang *et al*., 2002-2005) Although synthetic or chemical drugs are more efficacious and potent, as compared to phytomedicine, they are presenting a higher degree of side effects, money consumption and other health risks (Ali *et al*., 1986). Pakistan occupies a very distinctive situation in the list of developing countries because of medicinal plants which is recognized to variable edaphic conditions, climatic factors and rich flora of the different areas. Asides from this, the country is gifted by Allah with several ecological zones and topographical regions which contributes drastically to a rich biodiversity of Pakistan (Hussain *et al*., 2009 and Nisar *et al*., 2011). Pakistan is a country that having bio-diversity of medicinal plants. It is the observation that about 6000 plant species with prospective medicinal activities are broadly present in Pakistan, in which 600 to 700 species are being used for therapeutic purposes. (Haq *et al*., 1983) Approximately 60 % of the people of Pakistan depend on therapeutic plants for their drug-related needs (Hazrat *et al*., 2016). In our country, medicinal plants are initially used by Tibbi Dawa khanas (medical centers of local physicians known as Hakims). Sorry to say, very little consideration has been paid to the Ethnobotanical aspect of plants. As hakims are only related with the floral and vegetative parts of medicinal plants devoid of any hold to their botanical characteristics, or distribution in the various ecological zones of Pakistan, (Hazrat *et al*., 2011, Hamayun *et al*., 2003).

It is frequently thought that medicines from plants origins are safe and carry no or less risk to the user. But this is not the actual case, when poisonous plants are used by mistake or where herbal preparations are marketed with the accumulation of undeclared effective artificial substances. The use of man-made products should be governed and monitored by the same standard of safety and effectiveness as are required for current pharmaceutical producers (Hazrat *et a.l*, 2015). The main purpose of the present study to search for plants which can take alternate of the synthetics antibiotics and also to explore the plants of the study area..

MATERIALS AND METHODS

Data were collected from the study area by means of a semi-structured and close-ended questionnaire. Plants were collected from different hilly areas like Baba Bund, Hazar Nao, Kaparak, and Kwar Jabba of Agra valley and information in respect of various Ethnobotanical properties was gathered from local inhabitants of the study area. This was initially done by carrying the selected

plants to the mature men ranged from 30 - 75 years and above and occasionally to old women for assessment and specification. The questions from informants were asked in Urdu or else in their local language looking for the help of local support, concerning conventional uses of plants, their local names, allotment, morphology, and economical values. Each plant was photographed along with a voucher specimen for inhabitants and easy recognition in the herbarium. Collected plant species were dried, pressed, protect (poisoned), identified and finally were put up in the herbarium of the Department of Science and Information Technology Qurtuba University of Science and Information Technology Peshawar. Identification of the field collected remedial plants species were confirmed from Plant Taxonomy expert Dr. Ali Hazrat Department of Botany University of Malakand, and contrasting them with those in the different authentic Herbaria of Pakistan (Stewart., 1972 and Ali & Qasir., 2009).

Biological Activities

Out of these 70 medicinal plants four plants were collected about 4 or 5kg wet weight and were dried in shad, which were tested for different biological (antibacterial and antifungal) activities, in the Pakistan Council of Scientific and Industrial Research Labs. These plants are:

1-*Heteropappus altaicus* (Willd.)
Family Asteraceae
2-*Lespedeza cuneata* (Dum.Cours.)
Family Fabaceae
3-*Ficus sarmentosa* Buch. Ham. Ex Sm.
Family Moraceae
4-*Artemisia scoparia* Waldst. & Kit.
Family Asteraceae

Extract preparation

The plants materials were dried in shade, after complete drying, it is converted into powdered which can be stored. Each extraction of a medicinal plant depends on the nature of plant materials used to be extracted. All the plants parts were grinded by a simple grinder to obtain dried fine powder. About 10-20 g of the grinded fine powdered materials of each plant was taken in a flask. Methanol was added to each flask separately till the powdered materials were completely dipped in the methanol solvent. The flasks were kept in the oven on 60 C^o for about 2 days. Then filtered all the compounds soluble in methanol through the Whatman-1 filter paper. More solvent was poured into the used flask contents and the above procedure was repeat four times. Then the filtered solutions having plant compounds were subjected to the rotary evaporator.

The semisolid extract was removed from the flask, furthermore added to the china dish which was weighted already the extracts were dried out through a water bath at 45 C^o. When the extracts were dried completely, each extract was weighted again and the percentage production of the extract was determined.

Percentage extractive yield = Wt. of Extract / Wt. of actual Plant matter × 100

Media Used for culturing

Two types of media were used as a culture. Nutrient agar was used as a solid medium designed for the growth and culturing of all types of selected microbes. Nutrient broth liquid medium is used for inoculation, shaking incubation plus equality of microbes. As shown in tables 1 & 2.

Table1: Nutrient agar content composition used in culturing & applying test

S/No	Content	Composition (gram/letter)
1	**Agar**	15
2	**Grouse (Beef) extract**	1
3	**Extract of Gelatin**	5
4	**NaCl**	5
5	**Yeast extract**	2

Table: 2 Broth nutrient content composition used in standardization & inoculation

S/No	Contents	Composition (gram/letter)
1	**Grouse (Beef) extract**	1
2	**Extract of Gelatin**	5
3	**NaCl**	5
4	**Extract of Yeast**	2

Media preparation

Two types of nutrient mediums were prepared in distilled water. The reported quantity of nutrient agar 28 g/L and nutrient broth 13 g/L were taken in water (distilled) and then added to flasks and little amount of Broth were too added in the test tubes (15 cm^3). Everyone was closed with cotton wool plugs which were non-absorbent cotton wool, and then the cotton was applied by Aluminum caps. For sterilization all the materials listed below were put in autoclave i.e Blue tips micro pipettes, Flasks

having Nutrient agar**,** Flasks and test tubes having Nutrient broth**,** Petri dishes**,** Whitetip micropipettes, and Yellow tip micropipettes. Then sterilization was carried out in autoclave at standard pressure (1.5 pounds) and on temperature 121C^{o} for about 15 minutes. After cooling the sterilized media to 55C^{o}, the cotton plugs were removed, flame was applied to the mouth of the flasks over a Bunsen burner and the media was poured about 15 ml into each uncontaminated Petri dishes aseptically within a Laminar flow hood. The Petri dishes were kept back horizontally with reference to one hour until the medium becomes completely solidified. Then the Petri dishes be sited an upside-down situation in an incubator on behalf of 24 hrs to validate the contamination. After one day the uncontaminated Petri dishes were selected and then used in favor of bacteria and fungi culturing. Broth of flasks then used in favor of shaking incubation for bacteria and fungi whereas the test tubes media were applied on behalf of equivalence of culture.

Table: 3 Microbes used in Biological activities

Microbes	Nature	Source
Bacillus atrophaeus	Gram +ve Bacteria	PCSIR Lab Peshawar
Bacillus subtilis	Gram +ve Bacteria	PCSIR Lab Peshawar
Staphylococcus aureus	Gram +ve Bacteria	PCSIR Lab Peshawar
Escherichia coli	Gram -ve Bacteria	PCSIR Lab Peshawar
Citrobacter species	Gram -ve Bacteria	PCSIR Lab Peshawar
Pseudomonas aeruginosa	Gram -ve Bacteria	PCSIR Lab Peshawar
Salmonella typhi	Gram -ve Bacteria	PCSIR Lab Peshawar
Candida albicans	Fungus	PCSIR Lab Peshawar

Microbes used in current Biological activities

In the current biological activities, seven species of bacteria and one species of fungi were used. A total of seven bacterial species, three were Gram-positive while four were Gram-negative. The detail of bacterial strains and fungal species are given below.

Extracts used as a stock solution

The methanol extracts of each medicinal plant were diluted and adjusted by adding 6µl Dimethylsulfoxide (DMSO) solution to 1 mg of crude plant extract. On this way each 6µl of the DMSO solution contained 1 mg of the plant extract. This solution was used as a stock solution for testing the anti-microbial activities.

McFarland 0.5 turbidity standard

The pre-prepared 0.5 McFarland solution was used provided by the PCSIR Labs at Peshawar for turbidity standard. This solution contained 0.5 ml of 1.173% (wt / vol) barium chloride dihydrate solutions to 99.5 ml of 1% (vol / vol) sulfuric acid. (Fazal et al., 2011).

Disc Diffusion Susceptibility Method:

Antibiological activities for the selected medicinal plants species were evaluated through a paper diffusion manner followed the method already used via Fazal et al. (2011). The Bacterial culture was accustomed to 0.5 Mc Farland turbidity standards and transferred onto agar medium present in 15 cm diameter petri dishes. For the determination of anti-fungal activities of the medicinal plants Candida albicans was used. The concentration of C. albicans was adjusted as 10^{8}cfu/ml. The C. albicans culture was suspended in 0.9% normal saline sterile solution of and inoculated to agar Petri dishes (Fazal *et al*., 2012). Whattman-I filter paper discs having a diameter of 6-millimeter discs were soaked with standardized selected plant extract in the concentration of 1 & 2 mg dics^{-1} having DMSO volumes of 6 and 12 µl. All the bacterial cultures were put in incubation for about 24 hours at 37 C^{o}.

Stepwise Methodology of Biological activities Bioassay

The study of biological activities of the selected medicinal plants was a stepwise study regularly performed day by day. Here a brief description of procedures followed each day is given below.

Step 1st:

Nutrient agar medium having a concentration of 2.8 gram/100ml and Nutrient broth medium of 1.3 gram/100ml concentration. These concentrations were carefully weighed and dissolved in distilled water and poured in flasks. Some amount (approx. 10 milliliters) of broth was also added to the test tubes. For sterilization, every one of the materials was put in the autoclave. Sterilization was carried out in autoclave at standard pressure (1.5 pounds) and on temperature 121C^{o} for about 15 minutes. After cooling the sterilized media to 55C^{o}, the cotton plugs were removed, flame was applied to the mouth of the flasks over a Bunsen burner and the

media was poured about 15 ml into each uncontaminated Petri dishes aseptically in a Laminar flow hood. All the Petri dishes were then kept horizontally for one hour until the medium becomes completely solidified. Then Petri dishes were positioned in the upside-down condition in an incubator for the next 24 hours to check the contamination.

Step 2nd:

The microbial stock strands received from PCSIR labs at Peshawar were refreshing through takin germ-free inoculating loop on top of the nutrient agar dishes within a Laminar flow hood device. All the cultures were then put in incubation to be incubated for 24 hours at 37 C^{o}.

Step 3rd:

The re-fresh microbial cultures were then inoculated into the sterilized nutrient broth in flasks having about 25-30 ml broth media. The flasks containing microbial cultures in the nutrient broth were then incubated in a shaking incubator at 37 ^{0}C for 20 hours.

Step 4th:

The microbial cultures from the nutrient broth media of the flask were diluted and added to the test tubes and uniformed by matching with 0.5 Mc Farland turbidity standards. These standardized microbial cultures were taken about 50 µl and spread over the nutrient agar Petri dishes through sterilized glass spreader. For absorption, the dishes were then put in the refrigerator for about 20 minutes.

Step 5th Applying test:

All the Petri dishes from the refrigerator having homogeneous microbial cultures were placed in the Laminar flow hood. Whatman-I filter paper discs having a size of 6 millimeters in diameter were positioned on Nutrient agar medium having microbial cultures present in Petri dishes through uncontaminated forceps. After that, the methanol extracts of plants in different concentrations of the ratio of 1 & 2 milligram/disc in 6 and 12 µl volumes were then applied to each of the discs. Different antibiotics were also put on separate dishes for +ve control. All the cultures were then put in an incubator to be incubated for 24 hours at 37 C^{o}. The following antibiotics were used against different strands of Bacteria and Fungus.

1-*Escherichia coli* Amoxicillin
2-*Bacillus atrophaeus* Cephradine
3-*Bacillus subtilis* *Sulphamethoxazole/Trimethoprime*
4-*Staphylococcus aureus* Cephradine 30
5-*Citrobacter specie* Cephradine
6-*Pseudomonas aeruginosa* Tetracycline 30
7-*Salmonella typhi* Cephradine
8-*Candida albicans* Clotrimazole 50

Step 6th:

To evaluate the antimicrobial potential of each extract, a zone of inhibition was measured and recorded around each Whatman-I filter paper disc in mm. Pictures of the Petri dishes were taken through the digital cameras. All the tests were repeated for three times to remove the doubt. For the compilation of results, the mean ratios of inhibitory zones were taken.

RESULTS

The present study contains four different plant species that were screen for biological activities and their evaluation of the methanol extracts given below in table 4- 7 and Figure: 1-3.

Extractive Values of Selected Medicinal Plants

The methanol percentage extractive values of the four selected medicinal plants were carried out in the present study.The highest methanol extractive value was recorded for *Lespedeza cuneata* 15.4%, the next one was *Ficus sarmentosa* 10%, then followed by *Heteropappus altaicus* 8.7% and the least for *Artemisia scoparia* 8.1% table 4 and figure 1.

Table 4: Extractive values of selected medicinal plants

S. No	Plant Name	Sample code	% Methanol Ex.value
1	*Lespedeza cuneate*	A	15.4%
2	*Ficus sarmentosa*	C	10%
3	*Heteropappus altaicus*	B	8.7%
4	*Artemisia scoparia*	D	8.1%

Anti-bacterial activities of *Lespedeza cuneata*

Methanol 1 mg extract of the aerial parts of the *Lespedeza cuneata* (Dum.Cours.) show no activity against any type of selected species of bacteria, while 2 mg extract of the same plant also failed to show any biological activities table 6.

Anti-bacterial activities of *Heteropappus altaicus*

Methanol 1 mg extracts of the areal parts of *Heteropappus altaicus* (Willd.) Novopokr, Show best activity against *Citrobacter species* (18 mm)

followed by *B. atrophaeus* (17 mm), then *Bacillus subtilis* (9 mm), *Pseudomonas aeruginosa* (8 mm), lowest activity against *Escherichia coli* (7 mm), while there were no activities against *Staphylococcus aureus* and *Salmonella typhi*.

Methanol 2 mg extracts of the areal parts of *Heteropappus altaicus* (Willd.) Novopokr, Show best activity against *Citrobacter species* (23 mm), *B. atrophaeus* (23 mm), followed by *Bacillus subtilis* (14 mm), then *Pseudomonas aeruginosa* (10 mm), lowest activity against *Escherichia coli* (8.5 mm), while there were no activities against *Staphylococcus aureus* and *Salmonella typhi* figure 28.

Anti-bacterial activities of *Ficus sarmentosa*

Methanol 1 mg extract of the leaves and fruits of *Ficus sarmentosa* Buch. –Ham. Ex Sm. Show best activity against *B. atrophaeus* (21 mm) followed by *B. subtilis* (20 mm) then *Pseudomonas aeruginosa* (13 mm), *Escherichia coli* (12.5 mm) followed by then *Citrobacter species* (11 mm) and lowest activity against *Salmonella typhi* (9 mm) while there were no anti Bacterial activities against *Staphylococcus aureus*.

Methanol 2 mg extract of the leaves and fruits of *Ficus sarmentosa* Buch. –Ham. Ex Sm. Show best activity against *B. atrophaeus* (26 mm) followed by *B. subtilis* (22 mm) then *Citrobacter species* (16 mm), *Pseudomonas aeruginosa* (15 mm) followed by then *Escherichia coli* (13.5 mm) and lowest activity against *Salmonella typhi* (10 mm) while there were no activities against *Staphylococcus aureus* figure 29.

Anti-bacterial activities of *Artemisia scoparia*

The extract of the areal parts of plant species Artemisia scoparia Waldst. & Kit. were also extracted in methanol and were applied in two different concentrations against pathogenic species of some selected Bacterial strains. Methanol 1 mg extract of the areal parts of Artemisia annua L. show best result against Salmonella typhi (9 mm) and some activity against Escherichia coli (6.5 mm) while they show no anti Bacterial activities against any other selected species of bacterial strain.

After that the extract Artemisia scoparia Waldst. & Kit.was taken in higher concentration i.e. 2 mg. Methanol 2 mg extract of Artemisia scoparia Waldst. & Kit.shows somehow best results against Salmonella typhi (12 mm) followed by Pseudomonas aeruginosa (10 mm) and lowest activities against Escherichia coli (7 mm) while there were no activities against Staphylococcus aureus, B. atrophaeus, B. subtilis and Citrobacter species. Figure 30

Anti-fungal activities of the selected Medicinal plants

The Methanol extract of the four selected plant species was also applied against a single species of Fungus *Candida albicans* to check the anti-fungal activities of the plants species. Methanol 1 mg extracts of *Heteropappus altaicus* and *Ficus sarmentosa* shows same results against the fungus *Candida albicans* (7 mm) zone of inhibition to each, while *Lespedeza cuneata* and *Artemisia scoparia* show no results against *Candida albicans*.

Methanol 2 mg extracts of *Heteropappus altaicus* shows best result against *Candida albicans* (8.5 mm) and *Ficus sarmentosa* Shows results as (8 mm) of a zone of inhibition against *Candida albicans*. While the rest of plants i.e. *Lespedeza cuneata* and *Artemisia scoparia* show no antifungal activities in figure 31.

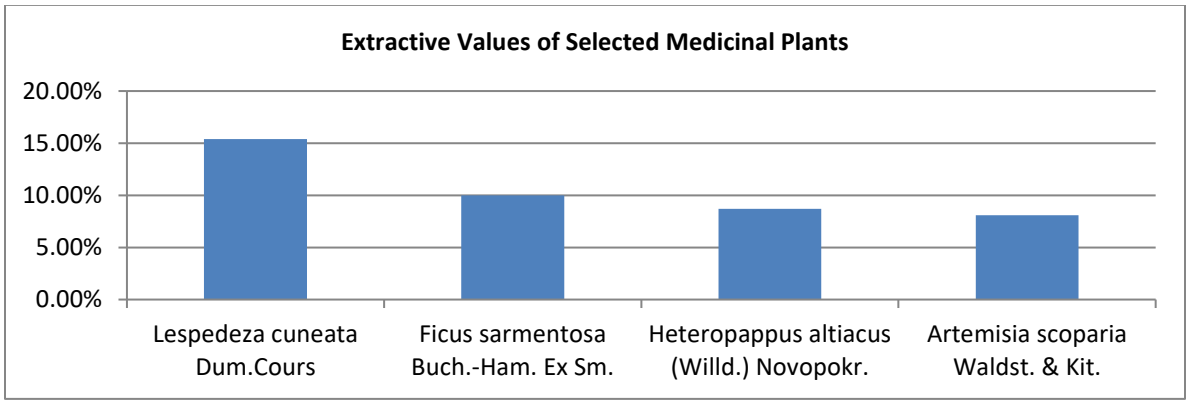

Figure 1: Percentage distribution of Extractive Values of Selected Medicinal Plants

Figure: 2 Nutrient Agar in Flasks

Figure:3 Packing for Sterilization

Figure: 4 Broth Medium in Cylinder

Figure:5 Specimen on water bath

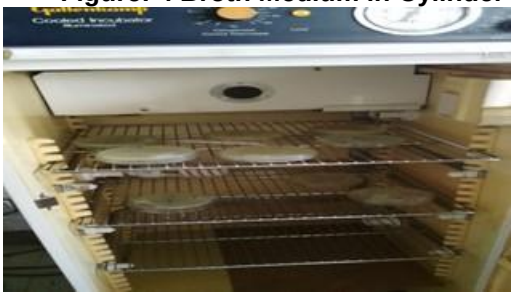

Figure: 6 Microbial species for refreshing

Figure: 7 Microbes refreshed

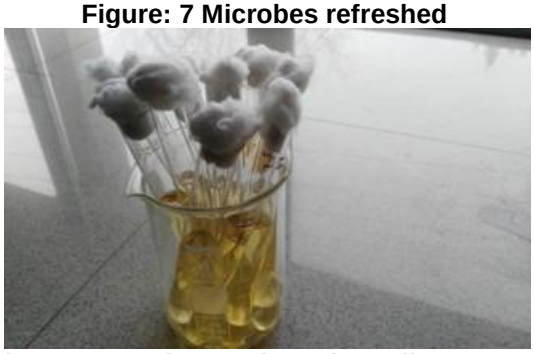

Figure:8 After sterilization in autoclave both the Agar Nutrient and Broth media

Figure: 9 Addition of DMSO to Extract

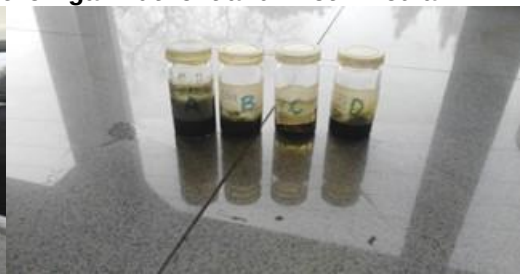

Figure: 10 DMSO added to each Extract

Figure:11 Microbial culture in Shaking incubator culture

Figure: 12 *Citrobactor species* refreshed

Figure: 13 *Bacillus atrophaeus* refreshed

Figure: 14 *E. coli* refreshed

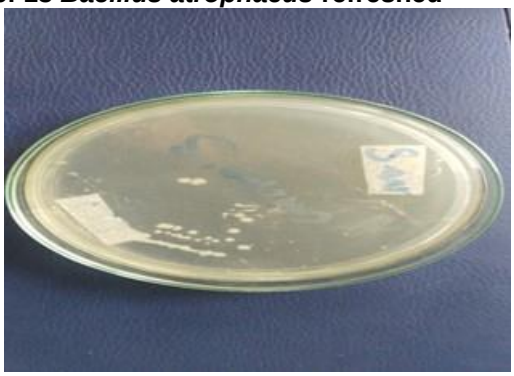

Figure: 15 Staphylococcus aureus refreshed

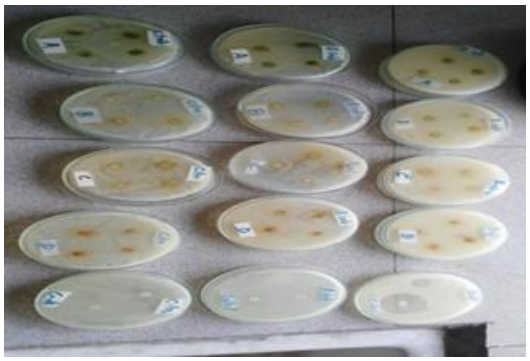

Figure: 16 after applying test

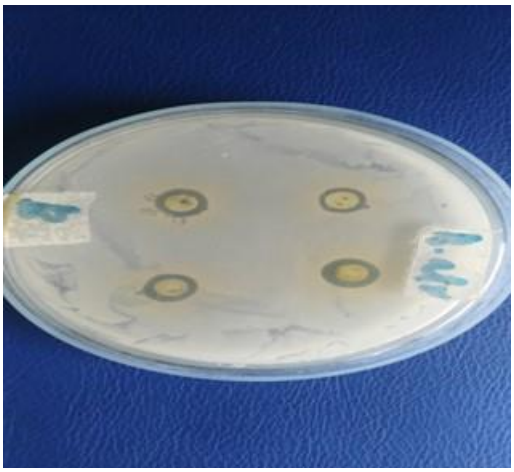

Figure: 17 Zone of inhibation by *B.atrophaeus*

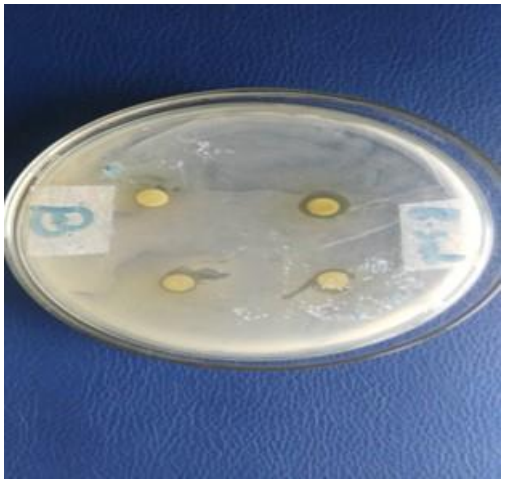

Figure: 18 Zone of inhibation by *B. subtilis*

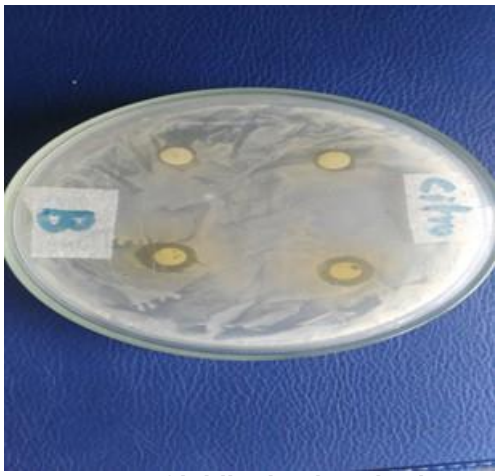

Figure: 19 Zone of inhibation by *Citrobacter* spp.

Figure: 20 Zone of inhibation by *B.atrophaeus*

Figure: 21 Zone of inhibation by *B. subtilis*

Figure: 22 Zone of inhibation by *Citrobacter spp*.

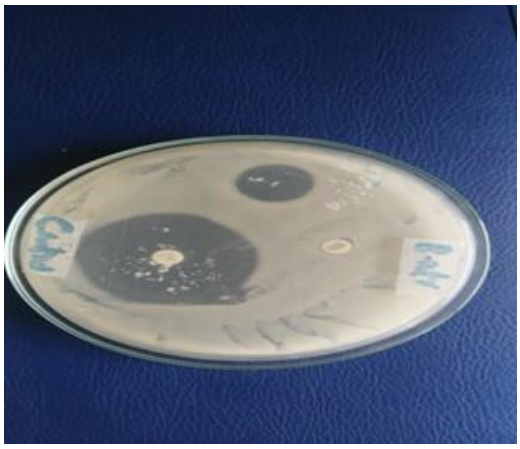

Figure: 23 Zone of inhibation by antibiotics

Figure: 24 Zone of inhibation by *E. coli*

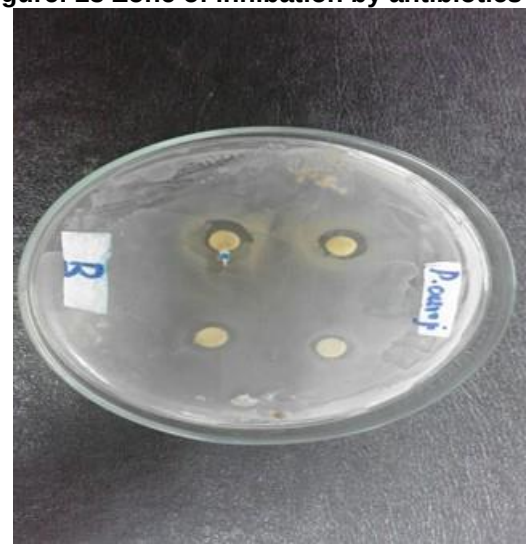

Figure: 25 Zone of inhibation by *Pseudomonas aeruginosa*

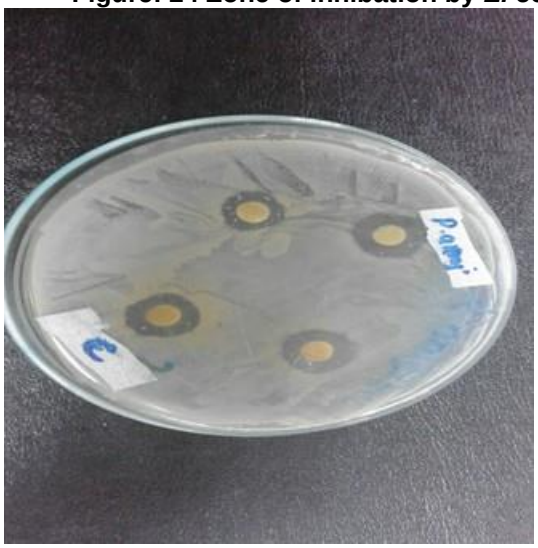

Figure: 26 Zone of inhibition by *Pseudomonas aeruginosa*

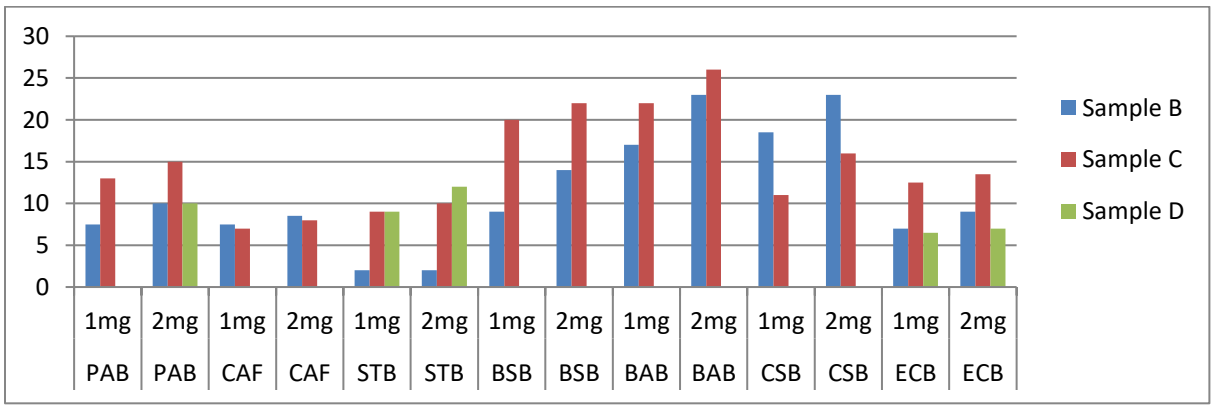

Figure: 27 Summary of Biological activities of the selected medicinal plants:
Sample A: *Lespedeza cuneata* (Dum.Cours.) Sample B: *Heteropappus altaicus* (Willd.) Novopokr.
Sample C: *Ficus sarmentosa* Buch. –Ham. Ex Sm. Sample D: *Artemisia scoparia* Waldst. & Kit

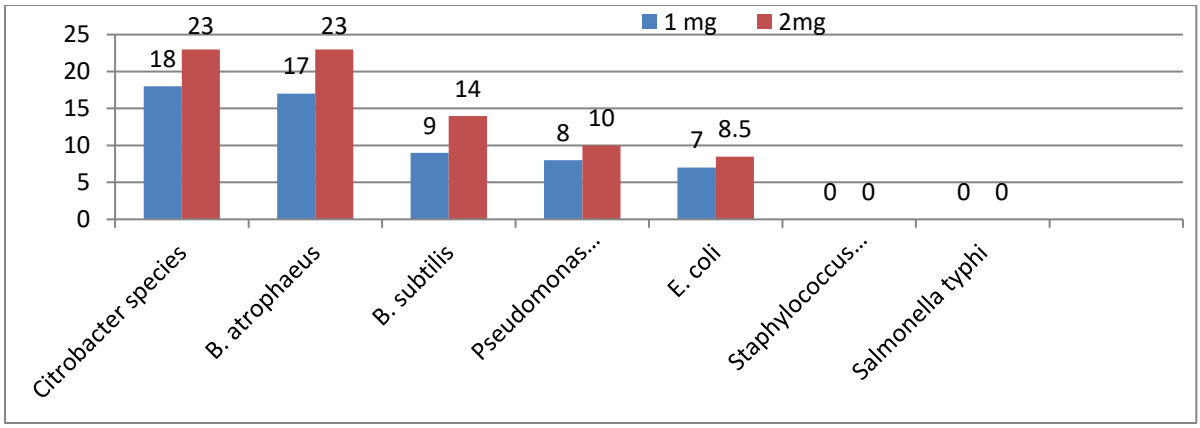

Figure: 28 Anti-Bacterial activities of *Heteropappus altaicus* (Willd.)Novopokr in (mm)

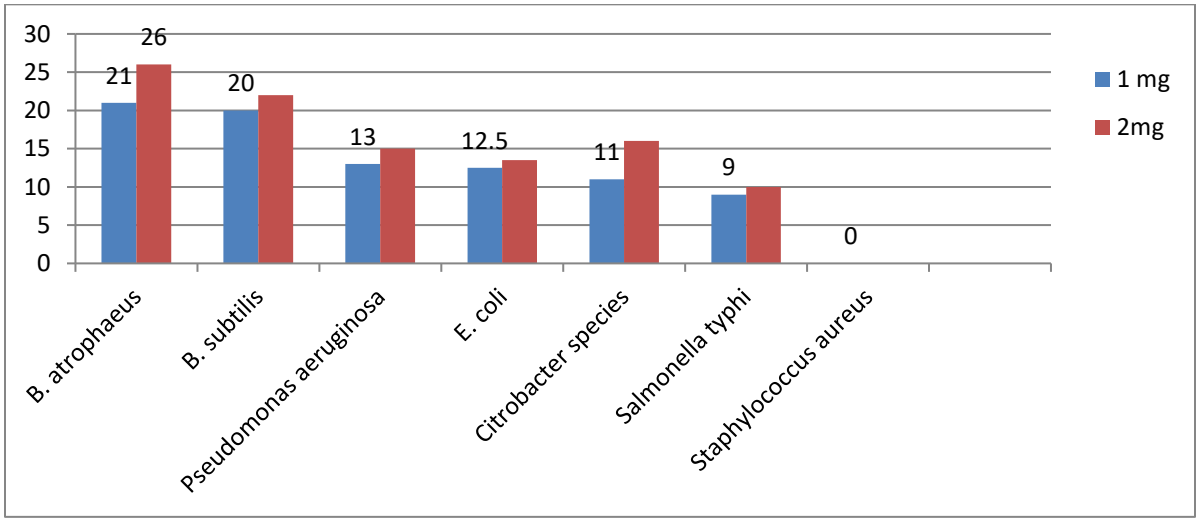

Figure 29: Antibacterial activities of *Ficus sarmentosa*

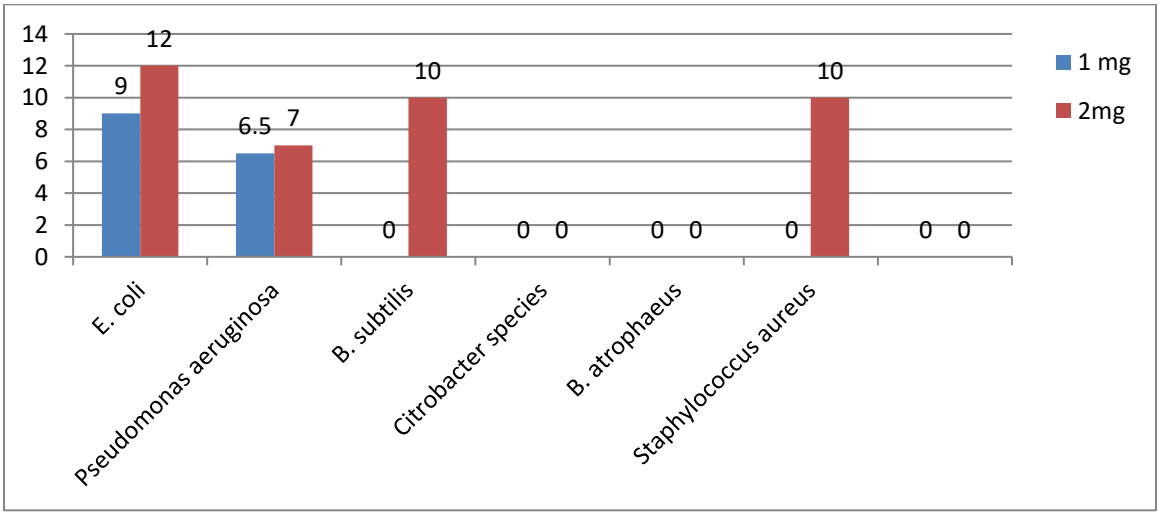

Figure 30: Antibacterial activities of *Artemisia scoparia*

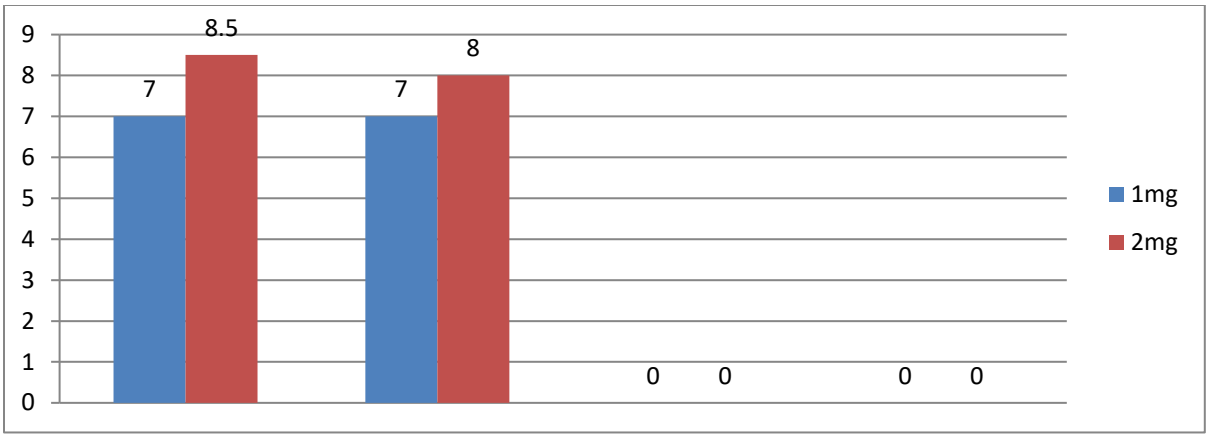

Figure 31: Antifungal activities the selected medicinal plants in (mm)

Table 5: Antimicrobial & Antifungal activities [zone of inhibition (mm)]

S. No.	Microorganisms used	Extract	Sample A mm			Sample B mm			Sample C Mm			Sample D Mm		
		Conc.	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1.	***Staphylococcus aureus*** (Bacteria)	1 mg	--	--	--	--	--	--	--	--	--	--	--	--
		2 mg	--	--	--	--	--	--	--	--	--	--	--	--
Con.	*Cephradine*	30 µg	16 mm											
2.	***Pseudomonas aeruginosa*** (Bacteria)	1 mg	--	--	--	7	7.5	8	13	12.5	13.5	--	--	--
		2 mg	--	--	--	10	9.5	10.5	15	15.5	14.5	10	9.5	10.5
Con.	*Tetracyclin*	30 µg	37 mm											
3.	***Candida albicans*** (Fungs)	1 mg	--	--	--	7.5	7	8	7	8	6	--	--	--
		2 mg	--	--	--	8.5	9	8	8	7	9	--	--	--
Con.	*Clotrimazole*	50 µg	36 mm											
4.	***Salmonella typhi*** (Bacteria)	1 mg	--	--	--	--	--	--	9	10	8	9	8	10
		2 mg	--	--	--	--	--	--	10	10.5	9.5	12	11	13
Con.	*Cephradine*	30 µg	21mm											
5.	***Bacillus subtilis(Bacteria)***	1 mg	--	--	--	9	8.5	9.5	20	21	19	--	--	--
		2 mg	--	--	--	14	13.5	14.5	22	23	21	--	--	--
Con	*Sulphamethoxazole/ Trimethoprime*	25 µg	40 mm											
6.	***Bacillus atrophaeus*** (Bacteria)	1 mg	--	--	--	17	16.5	17.5	21	22	23	--	--	--
		2 mg	--	--	--	23	23.5	22.5	26	25.5	26.5	--	--	--
Con	*Cephradine*	30 µg	--											
7.	***Citrobacter spp*** (Bacteria)	1 mg	--	--	--	18	18.5	19	11	11.5	10.5	--	--	--
		2 mg	--	--	--	23	24	22	16	15	17	--	--	--
Con	*Cephradine*	30 µg	--											
8.	***Escherichia coli*** (Bacteria)	1 mg	--	--	--	7	7.5	6.5	12.5	13	12	6.5	6	7
		2 mg	--	--	--	9	9.5	8.5	13.5	13	14	7.5	6.5	7
Con.	Amoxicillin	30 µg	27 mm											

Sample **A:** *Lespedeza cuneata* (Dum.Cours.) Sample **B**: *Heteropappus altaicus* (Willd.) Novopokr.
Sample **C:** *Ficus sarmentosa* Buch. –Ham. Ex Sm. Sample **D:** *Artemisia scoparia* Waldst. & Kit

Table 6: Means Results of Antimicrobial activities [zone of inhibition (mm)

	Microorganisms used	Extract	A mm	B Mm	C Mm	D Mm
1.	***Staphylococcus aureus***	1 mg	--	--	--	--
		2 mg	--	--	--	--
Con.	*Cephradine*	30 µg	16 mm			
2.	***Pseudomonas aeruginosa***	1 mg	--	8	13	--
		2 mg	--	10	15	10
Con.	*Tetracyclin*	30 µg	37 mm			
3.	***Candida albicans***	1 mg	--	7	7	--
		2 mg	--	8.5	8	--
Con.	*Clotrimazole*	50 µg	36 mm			
4.	***Salmonella typhi***	1 mg	--	--	9	9
		2 mg	--	--	10	12
Con.	*Cephradine*	30 µg	21 mm			
5.	***Bacillus subtilis***	1 mg	--	9	20	--
		2 mg	--	14	22	--
Con	*Sulphamethoxazole/ Trimethoprime*	25 µg	40 mm			
6.	***Bacillus atrophaeus***	1 mg	--	17	21	--
		2 mg	--	23	26	--
Con	*Cephradine*	30 µg	--			
7.	***Citrobacter***	1 mg	--	18	11	--
		2 mg	--	23	16	--
Con	*Cephradine*	30 µg	--			
8.	*Escherichia coli*	1 mg	--	07	12.5	6.5
		2 mg	--	09	13.5	7
Con.	Amoxicillin	30 µg	27 mm			

DISCUSSION

Four species of plant were selected for testing their Biological activities. The methanol extract of these plants were tested against 7 species of Bacteria i.e. Escherichia coli, Bacillus atrophaeus, Bacillus subtilis, Staphylococcus aureus, Citrobacter species, Pseudomonas aeruginosa and Salmonella typhi. One specie of Fungi was Candida albicans, to evaluate the anti-bacterial and anti-fungal potential of the selected medicinal plants. For this purpose Disc diffusion susceptibility method was used, previously used by Fazal et al. (2011). Out of the 4 tested plants Heteropappus altaicus (Willd.) Novopokr and Ficus sarmentosa Buch. –Ham. Ex Sm. show best results up to 26 mm of zone of inhibition against the selected pathogens of Bacteria Bacillus atrophaeus. While the plant Artemisia scoparia Waldst. & Kit. Showed partial biological activities against certain Bacterial strains i.e. Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi. The methanol extracts of only two plants were effective against Candida albicans. These plants were Heteropappus altaicus (Willd.) Novopokr and Ficus sarmentosa Buch. –Ham. Ex Sm. But the plant species Lespedeza cuneata (Dum.Cours.) were observed to have no such type of biological activities Table 6. For positive control some antibiotics were also used and their zone of inhibition was recorded. These antibiotics were Cephradine, Sulphamethoxazole/Trimethoprime, Tetracycline, Clotrimazole and Amoxicillin. All the antibiotics showed comparatively best results against each Bacterial strain, but Cephradine failed to show effectiveness against Bacillus atrophaeus and Citrobacter species.

CONCLUSION

The collected plants are *Lespedeza cuneate, Ficus sarmentosa*, *Heteropappus altaicus* and *Artemisia scoparia* were selected and the methanol extracts of these plants were tested for their biological activities, evaluated through Disc Diffusion Susceptibility Method. The activities of plant extracts were compared with those of standard antibiotic. The methanol extract was tested against 8 pathogenic species of microbes. The seven Bacterial species were *Bacillus atrophaeus*, *Bacillus subtilis*, *Escherichia coli*, *Citrobacter species*, *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Salmonella typhi*, while 1 species of fungus was *Candida albicans*. Their antibiotic activities were evaluated by measuring their zones of inhibition, inhibited by methanol extracts of selected plants. The major zone of inhibition was recorded for *Ficus sarmentosa*, followed by *Heteropappus altaicus*.

The minor activities were showed by *Artemisia scoparia* while *Lespedeza cuneata* exposed no such type of activities. It was concluded from the present study, that some plants extract having great potential against different types of pathogens. These plants are able to take the alternate position of synthetic antibiotics.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All the authors contributed in this article RU, AH, FH, GR designed and performleed the experiments and also wrote the manuscript. AR, II, WK and ZA performed experiments and data analysis. ZF, HS, TJ designed experiments and MAN reviewed the manuscript. All authors read and approved the final version.

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