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Reconnoitring Natural Antibacterial Appraisal of Medicinal Plants Extract Against Human Pathogen *Salmonella Paratyphi A* and *Salmonella Paratyphi B*

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Abstract: Regardless of the convenience of plentiful miscellaneous collection of synthetic products and high-throughput tactics for their biological testing, natural compounds twig at a major source for antimicrobial drug development. These compounds are exclusively treasured as they have endured natural assortment over time. In this study we concentrated on Ethnobotanical efficacy of Indian medicinal plants like *Ocimum sanctum*, *Phyllanthus emblica* and *Bryophyllum pinnatum* for defence against bacterial human pathogen *salmonella paratyphi A* and *salmonella paratyphi B*. Phytochemical screening of these plants was executed for constituents like alkaloids, flavonoids, tannins, steroids, glycosides, carbohydrates and aminoacids. The ethanol extract of these plants exhibited good activity against the human pathogens by agar well diffusion assay method and the MIC was recorded. Average mean zone of inhibition found by these plants ranged between 4 mm to 29 mm and 4 mm to 27 mm respectively for *salmonella paratyphi A* and *salmonella paratyphi B*. These results accomplish the antimicrobial potential of the medicinal plants and hence convey upkeep for the use of them in traditional medicine.

Keywords: Antimicrobial Activity, Ethnobotany, Inhibition, Human Pathogen, Medicinal Plants

1. Introduction

Today, the world faces an occurrence of innumerable infectious diseases. The increasing spread and drug resistivity of pathogenic strain have become a major threat to humanity. Side effects of several synthetic drugs and inadequate usages of antibiotics led to the drug resistivity of pathogenic strains. Prevention of resistant communicable disease has become the major issue of the healthcare sector at the global level since the late 20th century. Consequently, multiple drug resistivity reduced the efficacy of antimicrobial drugs and also making trouble in the treatment of patients [1].

Table 1. *Ayurvedic tradition of medicinal plants tested in the study.*

	Scientific Name	Common name	Part used	Ayurvedic approach
1	*Ocimum sanctum*	Tulsi	leaves	Treatment of bronchitis, arthritis. anticancer, antiasthmatic, [2]
2	*Bryophyllum pinnatum*	Kalanchoe pinnata, Pattharcatta	leaves	treatment of Kidney stones, Prevent alcoholic liver damage, [3]
3	*Phyllanthus emblica*	Amla	Fruit	anticancer, neuroprotective, Anti-cholesterol [4]
4	*Curcuma caesia*	Black turmeric	Fruit	antifungal, anti-asthmatic, [5]
5	*Datura stramonium*	Datura	Fruit, Flower	Analgesic activities, Treatment of Parkinson's disease.[6]
6	*Zingiber officinale*	Ginger, Adrak	Fruit	Improved the cellular metabolic activity, lowering clotting ability of blood. [7]

Despite the myriad of inventions in scientific and technological advancements in modern medicine, natural antimicrobial compounds are mostly preferred against synthetic drugs. Therefore, the utilization of medicinal plants emerges as a complementary and alternative healthcare remedy for several approaches as described in the (Table 1).

As the medicinal plant possesses thousands of natural compounds with different biological activity, their demand increases for primary treatment due to their higher efficiency and lesser side effects [8]. Medicinal value of these plants lies in these chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds [9]. Though many species are evaluated for their antimicrobial activity, a large part of them still shall undergo the antimicrobial assessment [10].

Recently, the medicinal plants started gaining momentum among researchers due to their antimicrobial properties. Mainly three tactics can be used to experiment with the antimicrobial activity of various plants against microbes. That can be finding plants with antimicrobial activities from popular herbal medicines or by searching antimicrobial plants, typical within a particular country or region, or by evaluating antimicrobial activities of different plants against specific pathogens [11]. In this chapter, we are heading ahead with the third approach i.e. exploring the antimicrobial activity of plant's crude extracts on human pathogen *Salmonella paratyphi A* and *Salmonella paratyphi B*.

Salmonella paratyphi is a common infectious agent and transmitted through fecal-oral route which is responsible for around 6000 deaths annually due to improper medication [12]. Three serotypes of Salmonella paratyphi are discovered that are *Salmonella paratyphi A, B* and *C* [13, 14]. *Salmonella Paratyphi A* and *Salmonella Paratyphi B,* collectively known as typhoidal Salmonella, are causal agents for a serious invasive (bacteraemic), intestinal lymphoid tissue infection, and gallbladder infection, sometimes fatal disease of humans called typhoid fever or paratyphoid fever. The mortality rate may vary between developed and developing countries. *Salmonella paratyphi* is Gram-negative, rod shaped, facultative anaerobe, non-encapsulated, non-spore forming, flagellated and motile bacteria [15]. These paratyphoid diseases are an important health issue in many countries including India, Pakistan, Peru, China etc. [16].

2. Methods

2.1. Plant Collection

Plant materials were collected in fresh state from the samarvani situated near dadranagar haveli, Gujarat (Figure 1). Plant materials were washed, air dried separately in shade and stored in the neatly labelled air tight plastic container till further use. The identification and authentication of plant material was confirmed at the Botany department; Gujarat college, Ahmedabad.

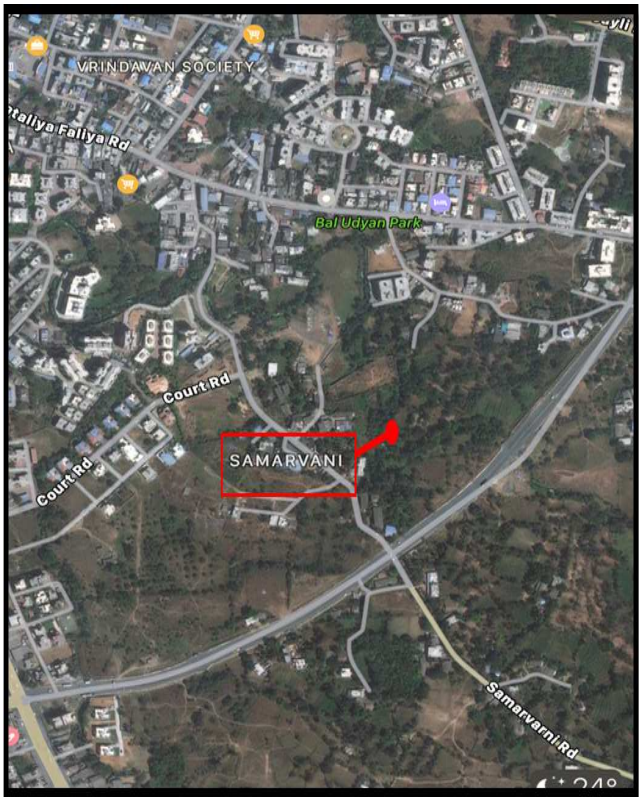

Figure 1. *Satellite image of sampling site for medicinal plants.*

2.2. Preparation of Plant Crude Extract

The fresh leaves of *Ocimum sanctum*, *Bryophyllum pinnatum* and the fruits of *Phyllanthus emblica* were collected and washed with the distilled water. Samples were amended into trifling pieces and crushed in a mechanical mortar. The extracts were filtered using a clean sterile muslin cloth, and then using whattman filter paper followed by using sterile membrane filters of 0.2 µ capacity. The filtrate was concentrated by evaporation using a water bath at 45°C [17]. The fresh crude extracts were stored in air tight containers. The extracts obtained were stored in a refrigerator at 4°C until required for further use [18].

2.3. Preparation of Plant Aqueous Extract

5 ml of plant extract were mixed in 25 ml of distilled water which mixed thoroughly and filtered through a Whatman filter paper followed by sterile membrane filters of 0.2 µ capacity. The sterility of the extracts were maintained meticulously [19].

2.4. Preparation of Plant Methanol Extract

Plant methanol extract was prepared with 200 g fresh plant leaves which were evaporated to dryness and dissolved in 100 ml of 100% methanol (MeOH) overnight. Extracts (50

mL) were then transferred to clean vessels, evaporated to dryness, and redissolved in dimethyl sulfoxide (DMSO) to harvest an ultimate concentration of approximately 10 mg/ml [20].

2.5. Phytochemical Analysis

Freshly prepared extracts were exposed to standard phytochemical consideration to find the presence of the following phyto constituents phenols, flavanoids, alkaloids, glycosides, tannins, saponins and steroids [21].

2.6. Test Microorganisms

Pure isolates of *salmonella paratyphi A* and *salmonella paratyphi B.* were obtained from the Microbiology department, Gujarat College, Ahmedabad, Gujarat, India and stored on a nutrient agar slants at 4°C until needed. The clinical isolates were biochemically and serologically categorised by standard approaches.

2.7. Preparation of Inoculum

Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient broth (NB) for test organisms and incubated with agitation for 24 h at 37°C. To 5 ml of NB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600 nm which is equivalent to 106– 108 CFU/ml [22].

2.8. Determination of Antimicrobial Activity

Antimicrobial activity was distinguished by the agar well diffusion method [23], as implemented earlier (Ahmad & Beg, 2001)[24] was used; 0.1 ml of diluted inoculum (106-108 CFU/ ml) of test organism was spreaded on nutrient agar plates with sterile glass spreader by spread plate technique. Wells of 7 mm diameter were perforated into the agar medium which was auxiliary occupied with 0.3 ml of plant extract, and allowed to diffuse at room temperature for 2 h. and then the plates were incubated for 24-48 hours at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. The experimentation was carried out in duplicate and the mean of the diameter of the inhibition zones were calculated.

2.9. Minimum Inhibitory Concentration (MIC)

Equipped extracts were re-formed, and three fold dilution were prepared in covenant to antimicrobial sensitivity test. Serial dilutions were made from the extract using the same solvent as per extraction (aqueous and methanol). 8 ml of sterile nutrient broth for test organisms were inoculated with 2-3 drops of inoculum after addition of 0.8 ml of extract of each concentration, thus making three tubes of each extract for an organism. Broths free of organism and extract with the other inoculated organism were used as controls. These nutrient broths were incubated at 37°C for 24 hours. The lowest concentration that did not permit any visible growth was reflected as MIC [25].

Table 2. *Phytochemical profiling of medicinal plants.*

Phytochemicals analysis						
				Results for Plants		
No.	**PHYTOCHEMICAL**	**TEST**	**OBSERVATION**	***Ocimum sanctum* (Tulsi)**	***Phyllanthus emblica***	***Bryophyllum pinnatum***
1	Alkaloids	tanic acid test	white ppts	-	+	+
2	Glycosides	keller-killani's test	orange red color	-	-	+
3	Flavonides	lead acetate test	yellow color	+	+	+
4	Flavonides	alkali test	yellow color ppts	+	+	-
5	Carbohydrates	benedict's test	orange red ppts	+	-	+
6	Carbohydrates	molisch test	violet ring at the junction	+	+	+
7	Phenols	ferric chloride test	bluish black color	+	+	+
8	Steroides	salkowski's test	golden yellow color	+	+	+
9	Saponins	foam test	foam formation	+	+	+
10	Proteins	Niunhydrin test	Blue color	+	+	+

3. Results

3.1. Preliminary Phytochemical Assessment

The results of the preliminary phytochemical assessment of the various medicinal plants extract (i.e. leaf, flower and fruit extracts) are described in Table 2. Which evidently demonstrates numerous secondary metabolites are present in tested plants. Several biochemical compounds like tannins, triterpenoids, alkaloids, cardiac glycosides, flavonoids, and steroids were present in the extract of the medicinal plants which directly or indirectly provide defence against *salmonella paratyphi A* and *salmonella paratyphi B.*

3.2. Antibacterial Assay

In this study, the antibacterial efficiency of different extracts (crude, methanol and aqueous) of medicinal plants *Ocimum sanctum*, *Phyllanthus emblica*, *Bryophyllum pinnatum*, *Curcuma caesia, Zingiber officinale* and *Datura inoxia* (i.e. leaf, flower and fruit extracts) were tested against two human pathogens (*salmonella paratyphi A* and *salmonella paratyphi B.*) by using the agar well diffusion method. The results of the antimicrobial effects of different parts (i.e. leaf, flower and fruit) of the medicinal plants with different solvent extracts are presented in Figure 2, Figure 3, Figure 4. Where it shows maximum inhibition by methanol

extract and the average zone of inhibition found by *Ocimum sanctum* is 29 mm and 27 mm for *salmonella paratyphi A* and *salmonella paratyphi B.* respectively. Where methanol extracts of *Phyllanthus emblica* gives average zone of 7 mm against tested organisms and *Bryophyllum pinnatum* proves to be effective against strain of *salmonella paratyphi B* which gives the zone of 12 mm in the methanolic extracts. Although *Curcuma caesia, Zingiber officinale* and *Datura inoxia* were not capable of defending that pathogens and produces minute zone of inhibition. Comparative study of crude, aqueous and methanolic extracts are shown in Figure 3, Figure 4.

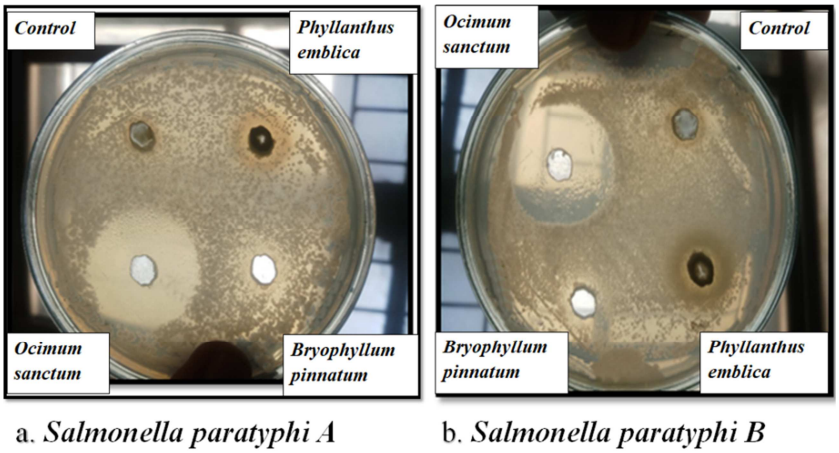

Figure 2. *Zone of inhibition by methanolic extract of medicinal plants against Salmonella Paratyphi A and Salmonella Paratyphi B.*

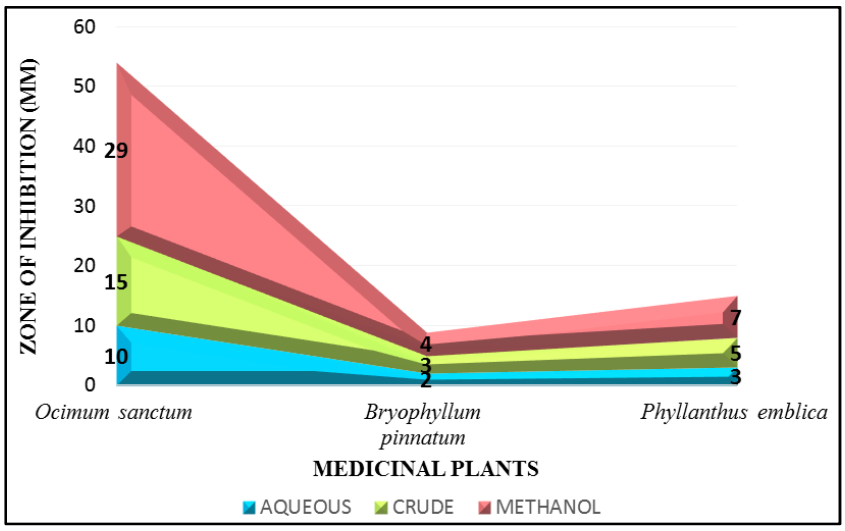

Figure 3. *Average zone of inhibition by medicinal plant extracts against salmonella paratyphi A.*

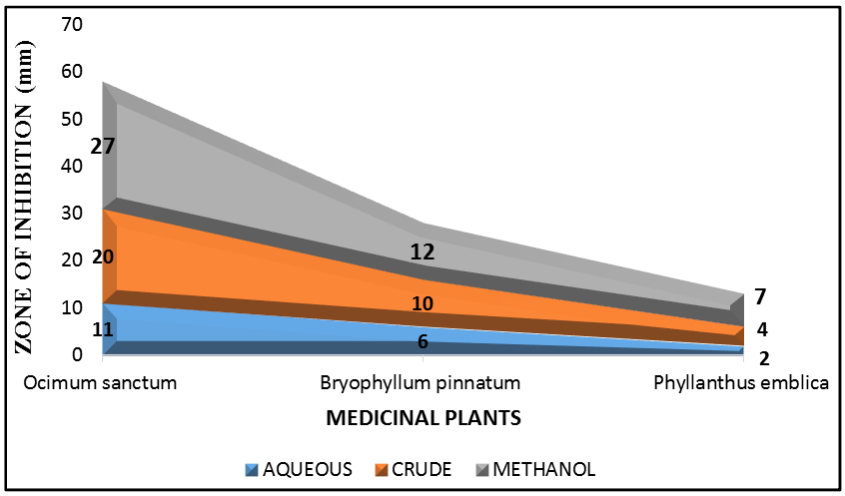

Figure 4. *Average zone of inhibition by medicinal plant extracts against salmonella paratyphi B.*

3.3. Determination of Minimum Inhibitory Concentration (MIC) of Medicinal Plant Extracts

The lowermost concentration at uppermost dilution of medicinal the plant extract obligatory to inhibit evident growth of the tested pathogenic bacteria was preferred as the MIC (table 3) MIC of various plant extracts of a specific plants varies against various strains of *salmonella* species. MIC values attained from leaf extracts of *Ocimum sanctum* was found comparative good than other extracts MICs. (Figure 5, Figure 6)

Table 3. *The minimum inhibitory concentration of medicinal plant extracts against S. paratyphi A and B.*

	PLANTS	EXTRACT	*S. paratyphi A*	*S. paratyphi B*
MIC concentration mg/ml				
1	*Osmium sanctum*	Methanol	0.33	0.35
		Crude	0.85	0.89
2	*Phyllanthus emblica*	Methanol	4.00	4.97
		Crude	4.60	4.71
3	*Bryopyllum pinnatum*	Methanol	6.70	6.80
		Crude	7.80	7.85

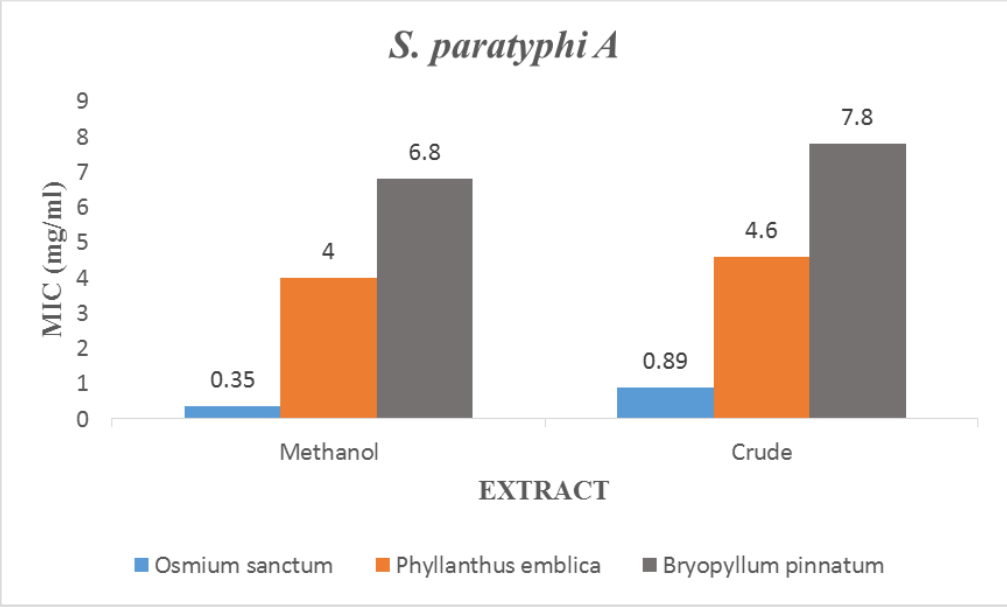

Figure 5. *MICs (mg/mL) of the tested extracts against salmonella paratyphi A.*

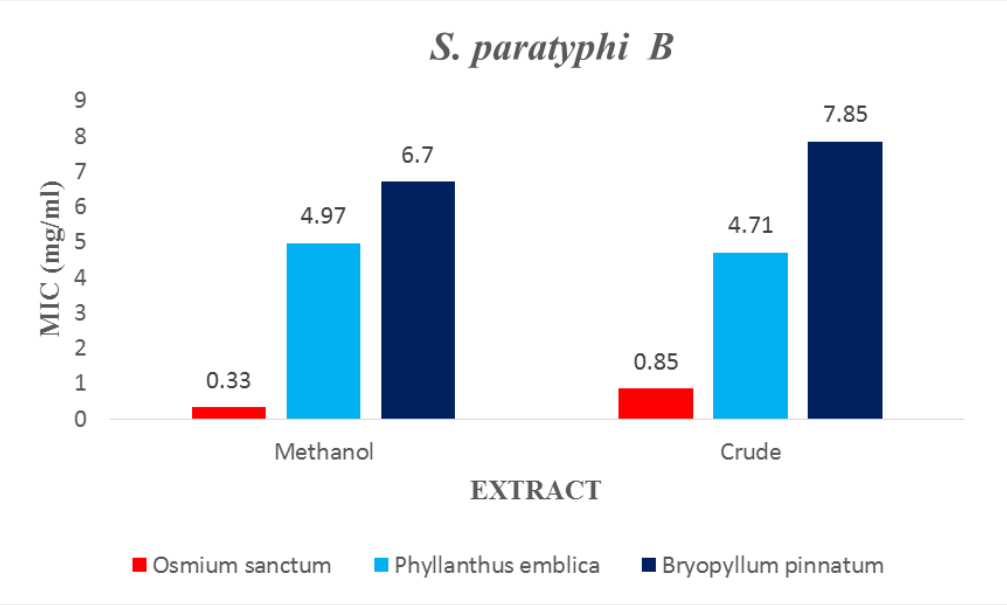

Figure 6. *MICs (mg/mL) of the tested extracts against salmonella paratyphi B.*

4. Discussion

In India, a miscellaneous flora of medicinal plants are grown-up naturally. In the present research, we have investigated the antibacterial activity of six naturally growing plants: *Ocimum sanctum, Bryophyllum pinnatum* and *Phyllanthus emblica* Curcuma caesia, Zingiber officinale and Datura inoxia (i.e. leaf and fruit extracts). The biochemical activity of these plants were tested against known human bacterial pathogens *Salmonella Paratyphi A* and *Salmonella Paratyphi B*.

Excessive use of antibiotics and chemical synthetic drugs frequently caused development of drug resistance in pathogenic strains and therefore search for new biomedicine is requisite [26]. In this problematic situation plants continue to be a rich source of therapeutic drugs and Use of plants as a source of medicine has been an antediluvian exercise and have a significant component of the health care system in India but only a few of them have been studied for their antimicrobial activities [27].

In this study agar well diffusion assay method was used to detect zone of inhibition. Later on based on their antibacterial properties three medicinal plants were selected for further study (i.e. *Ocimum sanctum*, *Phyllanthus emblica* and *Bryophyllum pinnatum)*. From these selected plants the zone of inhibition varies within the ranges of 4 mm to 29 mm. The highest zone of inhibition was found against *Salmonella Paratyphi A* (29 mm) and *Salmonella Paratyphi B* (28 mm) respectively from methanol extract of *Ocimum sanctum.* Which is comparative with the positive control of antibiotic streptomycin 15 mm. thus it clearly indicates that the selected bacterial strains of *salmonella* were resistant to that antibiotic and gives more effective results with methanol extract of the selected naturally growing plants.

The difference in antimicrobial activity was significant when compared with other plants extracts like *Momordica charantia* shows 27 mm diameter of zone against S. Paratyphi A, *Rubia cordifolia* and *Garcinia indica* also showed 25 mm activity against salmonella strains (25 mm diameter of zone). The methanol extract of this plant presented similar antibacterial activity against S. typhi and S. Paratyphi A (22 mm) respectively, extracts from leaf of *Azadiracta indica* showed the highest activity against S. Paratyphi A (20 mm) (Pasha, Sayeed, ALI, & KHAN, 2009). The extracts of *Hemidesmus indicus (Linn.) Schult* (8 mm), *Mangifera indica* (9 mm), *Wrightia tomentosa* (12 mm) and *Xanthium strumarium* (12 mm) also showed comparative a lesser amount of antibacterial activity against *Salmonella Paratyphi A* [28].

Phyllanthus emblica (7 mm) and *Bryophyllum pinnatum* (4 mm) were less effective where visible growth inhibition is so concerned with the bacterial strain as compared to *Ocimum sanctum* but still they inhibited the growth of microorganisms up to certain level (Figure 3, Figure 4).

MIC was determined against selected pathogenic bacteria to quantify the activity of these plant extracts. *Ocimum sanctum* gave MIC values of 0.33 mg/ml and 0.85 mg/ml respectively, for methanol and crude extracts against *S. paratyphi A*, also 0.35 mg/ml and 0.89 mg/ml respectively, for methanol and crude extracts against *S. paratyphi B* which is significant than other two plants and the positive control of antibiotic tested for MIC in the study. Where there is much study done for their MIC against *salmonella typhi* nevertheless no such efficient medicinal plants are reported for MIC against human pathogen *salmonella paratyphi A* and *salmonella paratyphi B*.

Phytochemical profiling of plant extracts showed that they were also rich in phytosteroles, flavonoids, alkaloids, steroids, phenols, amino acids, proteins, carbohydrates, tannins and saponins. Gowri & Vasantha, 2010 reported that these phytochemicals have various properties like anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Assessments with relevant data from literature indicate that, according to the methodology embraced in studies on antimicrobial activity, the most diverse results can be obtained. Plant extracts have shown inhibitory effect on the growth of the bacteria studied. It is therefore recommended that the natural surrounding flora and the number of the active antibacterial principles involved in each plant extract can be studied in detail [29].

5. Conclusion

This study determines the antimicrobial activity of the crude extracts, aqueous extract and methanol extract of medicinal plants (*Ocimum sanctum, Bryophyllum pinnatum* and *Phyllanthus emblica*). The results discovered that the bioactive compounds present in these medicinal plants and methanolic extracts were active against Gram-negative human pathogens *salmonella paratyphi A* and *salmonella paratyphi B*. However the aqueous and crude extracts showed fewer inhibitions against both human pathogen. The result recommends that the methanolic extracts can be used as antibacterial supplements in the expansion of herbal medicine formulations. Though, additional study and phytochemical investigation are still required to isolate biologically active compounds from these natural crude extracts.

Authors' Contributions

JR and CKJ planned the research. JR, AD, JH, AP carried out the experiment and analysis under the guidance of CKJ. JR wrote the manuscript with acute contribution of AR and NJ. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Ethics Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest

All the authors do not have any possible conflicts of interests.

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