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Antibacterial activity of *Asparagus racemosus* willd. and *Ocimum gratissimum* L. in urinary tract infection from in and outdoor patients of Duta Deepti Satsang Charitable Hospital, Deoghar, Jharkhand, India

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Abstract
Asparagus racemosus Willd. (Asparagaceae) and *Ocimum gratissimum* (Lamiaceae) are commonly used traditional medicinal plants in Jharkhand for urinary tract infections (UTI). In the present investigation, an attempt was made to study the antimicrobial activity of *A. racemosus* and *O. gratissimum* against three predominant UTI bacteria obtained from in and outdoor patients of Duta Deepti Satsang Charitable Hospital, Deoghar, Jharkhand, India. The antibacterial activity of plant extracts was screened by the disc diffusion method of Kirby-Bauer sensitivity test against three predominantly occurred UTI bacteria *viz*., *E. coli* (DDSCH54), *Klebsiella pnemonae* (DDSCH62) and *Proteus mirabilis* (DDSCH72). Among all the extracts, the root ethanol extract of *A. racemosus* was found to exhibit better antimicrobial activity against *E. coli* (DDSCH54) and *K. pnemonae* (DDSCH62) at 20 mg/ml concentration whereas in case of *O. gratissimum*, the seed methanol extract showed more antimicrobial activities against all the three UTI bacterial isolates as compared to control (nitrofurantoin). Root ethanol extract of *A. racemosus* exhibited maximum inhibition 9.33±0.88 mm against *E. coli* (DDSCH54) and 9.33±0.66 mm against *K. pnemonae* (DDSCH62). However, the seed methanol extract of *O. gratissimum* inhibition 9.33±0.22 mm against *E. coli* (DDSCH54); 9.4±0.3 mm and 9.4±0.6 mm against *K. pnemonae* (DDSCH62) and *P. mirabilis* (DDSCH72) respectively. The minimum inhibitory concentration (MIC) revealed no inhibitory effect at 1.25 and 2.5 mg/ml concentration of both the ethanol extract against test organisms. The ethanol root extract of *A. racemosus* showed inhibition at 0.156 mg/mL and seed methanol extract of *O. gratissimum* exhibited inhibition zones at 0.078 mg/mL as compared with ethanol extract. From overall MIC's observation, methanol extract of *O. gratissimum* at 20.0 mg/ml concentrations showed maximum zone of inhibition for all the test organisms. This investigation proved the efficacy of both the traditional medicinal plants for antibacterial activity against predominate UTI bacteria.

Keywords: antimicrobial activity; traditional medicinal plant; *asparagus racemosus; ocimum gratissimum*; minimum inhibitory concentration

Introduction
Urinary tract infections (UTI) are one of the most common human bacterial infections both in the community and hospital settings. UTI affects people of all age groups, being more common in women. A large majority of such infection are caused by Enterobacteriaceae, mainly *E. coli* which are known to acquire drug resistance easily. In most of the cases there is a need to start a prophylactic therapy before culture and sensitivity results are available (Joseph and Raj 2010) [1]. Therefore, it is very much pertinent to look after the plant based traditional medicines to overcome the drug resistance issues etc. Medicinal plants are the important natural source of different bioactive and phytochemicals compounds, widely used in traditional healthcare practices by ethnic communities throughout the globes (Sarma *et al* 2017; Tanti *et al* 2010) [2, 3]. Alkaloids, steroids, tannins, phenolic compounds, etc. that are secondary metabolites in plants are the principal phytochemicals with different medicinal properties (Rai *et al* 2008; Pullaiah 2006; Viswanad *et al* 2011) [4, 5, 6]. There is an increasing demand for plant based pharmaceuticals throughout the globe. Very little medicinal plants have been explored so far scientifically and still there is large scope to characterize more which are still unexplored to fulfil the demands of the ever expanding pharmaceutical industries (Baruah *et al* 2017; Das *et al* 2020) [7, 8]. There is always high demand for the investigation of traditional medicinal plants for exploiting in pharmaceutical activities because of less toxicity and cost effective and therefore attention has been made for utilization of antimicrobial activity of natural origin and other bioprospection (Bhalodia and Shukla 2011) [9]. *Asparagus racemosus* Willd. and *Ocimum gratissimum* L. are widely used in the traditional healthcare practices mostly by the ethnic communities of Deoghar District of Jharkhand and therefore, an attempt was made to study an antimicrobial activity from root extract of *A. racemosus* and seed extract of *O. gratissimum* against few predominant UTI bacteria.

Materials and Methods
Collection of the plant material and preparation of the plant extract
Based on secondary information and interaction with the local tribes of Doeghar, Jharkhand (India), two plants *i.e.*, *Ocimum basilicum* (Lamiaceae) and *Asparagus racemosus* (Asparagaceae) were selected for antibacterial analysis. Fresh roots of *A. racemosus* and seed of *O. gratissimum* were collected. Collected samples were air-dried at room temperature for about one month. The dried samples were then coarsely powdered in a mechanical grinder.

Microbial strains

For antibacterial activity, three bacteria *viz.*, *Escherichia coli* (DDSCH54), *Klebsiella pnemoniae* (DDSCH62) and *Proteus mirabilis* (DDSCH72) that showed highly resistant against the panel of antibiotics as well as predominately occurred in urinary tract infection from in and outdoor patients of Duta Deepti Satsang Charitable Hospital, Deoghar, Jharkhand, India were selected. Bacterial strains were maintained in MHA (Muller Hinton agar medium) at 4°C for further experiments.

Solvent extraction

The solvent extraction was done using soxhlet method. 10 gm each of coarsely powdered root and seed samples were extracted separately in 100 ml of ethanol and methanol using the soxhlet apparatus for 24 hrs. The extracts were then filtered with Whatman filter paper. The filtrate was evaporated in a hot air oven at 45ºC. The crude extract thus obtained was lyophilized. The dried extract was then stored at 4°C for further use (Roy *et al* 2017) [10].

Preparation of the standard concentration of the plant extract

Stock solutions of the extracts were prepared by dissolving the dried extract in 10% DMSO (Dimethyl sulfoxide) at the concentration of 200 mg/ml (Parekh and Chanda 2006; Jouda *et al* 2016) [11, 12]. From the stock solution, different volumes of the extracts were prepared to get the final amount of 20, 10, 5.0 and 2.5 mg/mL concentration. Sterile filter paper discs were loaded with different concentrations of the extracts and allowed to dry at room temperature under aseptic conditions.

Preparation of the microbial suspension

The bacterial slants were prepared in Mueller Hinton Agar (MHA) medium in test tubes and stored at 4°C. Active cultures were prepared by transferring a loopful of cultures in Mueller Hinton broth medium incubated at 37° C for 24 hrs.

Antibacterial activity assay

The antibacterial activity of plant extracts was screened by the disc diffusion method of Kirby-Bauer sensitivity test (Bauer *et al* 1966; Murray *et al* 1995) [13, 14]. The MHA plates were spread uniformly with 100 µl of bacterial cultures (108 CFU/ml) of all the bacterial strains. They were allowed to dry for 10 min. Then, the discs (0.6 cm) were loaded with 20 µl of 20, 10, 5 and 2.5 mg/mL extract respectively. The loaded discs were allowed to remain for diffusion for 30 min at room temperature. Nitrofurantoin disc (30 µg, Hi-Media) was used as a positive control. The plates were incubated at 37°C for 24-48 hrs. Zone inhibition formed around the discs were measured in millimeters and recorded. The experiment was repeated twice with three replicas per experiment.

Determination of MIC for antibacterial activity

The minimum inhibitory concentration (MIC) was determined in 96 well microtitre plates following the broth microdilution method based on the Clinical Laboratory Standard Institute (CLSI, 2009) [15]. Two-fold serial dilution of the plant extracts (both root and seed extracts prepared in ethanol and methanol separately) were prepared. From the stock solution (2.5 mg/ml) of previously studied zone inhibition test, six different dilutions were prepared as 1.25, 0.625, 0.3125, 0.156, 0.078 and 0.039 mg/ml respectively. 50 µl of MH broth was dispensed into 96 well plates vertically from WA (first well) to WH (eighth well). Then, 50 µl of 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 mg/ml extracts were poured in each well from WA to WF. In the seventh well (WG), Nitrofurantoin was used in place of the plant extract as positive control and the eighth well (WH) was used as negative control which consists of MH broth and extract. 50 µl of bacterial suspension (1×10^5 CFU/ml) was inoculated in each of the wells (from Well A to Well G). The microdilution plates were incubated at 37º C for 24 h. After incubation, the bacterial growth was observed by taking absorbance at 405 nm (Taye *et al* 2011) [16]. The presence of turbidity was considered when the difference of OD value (after incubation-before incubation) of the tested extracts was more than the control (broth + extract).

Results

Description of the experimental plants

Asparagus racemosus belongs to the family Asparagaceae, commonly found in Sri Lanka, India, and other parts of Asia including the Himalayan Mountains. It is a woody climber that reaches a height of 1-2 m and prefers the shade to take root in the piedmont's gravelly, rocky soils. The leaves are tiny and uniform, like pine needles, and the flowers are white with small spikes. The most common species of *Asparagus* grown in India is *A. racemosus*, which is used in traditional Indian medicine (Fig. 1A). *Ocimum gratissimum* commonly known as Ram Tulsi belongs to family Lamiaceae. It is a perennial herbaceous plant grows about 1–2 feet long with a woody base. The leaves are usually broad and ovate in shape 5–13 cm in length, 3–9 cm in width. The leaves are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, simple and decurrent at base with a coarsely crenate, serrate margin, pubescent and dotted on both sides, and measure over 105 cm in length. Covering and glandular trichomes can be seen on the leaves. On the upper surface, stomata are scarce or absent, while on the lower surface, they are abundant. Ordinary trichomes are few, and the long ones (up to six celled) are mostly found on the margins; the two celled ones are mostly found on the lamina. Racemes can be up to 18 cm long and petioles can be up to 6 cm long. The peduncles have a dense layer of pubescence on them. Calyx is up to 5 mm long, campanulate, and 5–7 mm long, and the colour varies from greenish-white to greenish-yellow.

This plant has immense medicinal uses like chemopreventive, ant carcinogenic, free radical searching, radio defensive, and various other pharmacological uses. It delivers different bioactive constituents which are utilized usually as sustenance added substances, nourishment colorants, pharmaceuticals, pesticides, aromas etc. (Fig.1B)

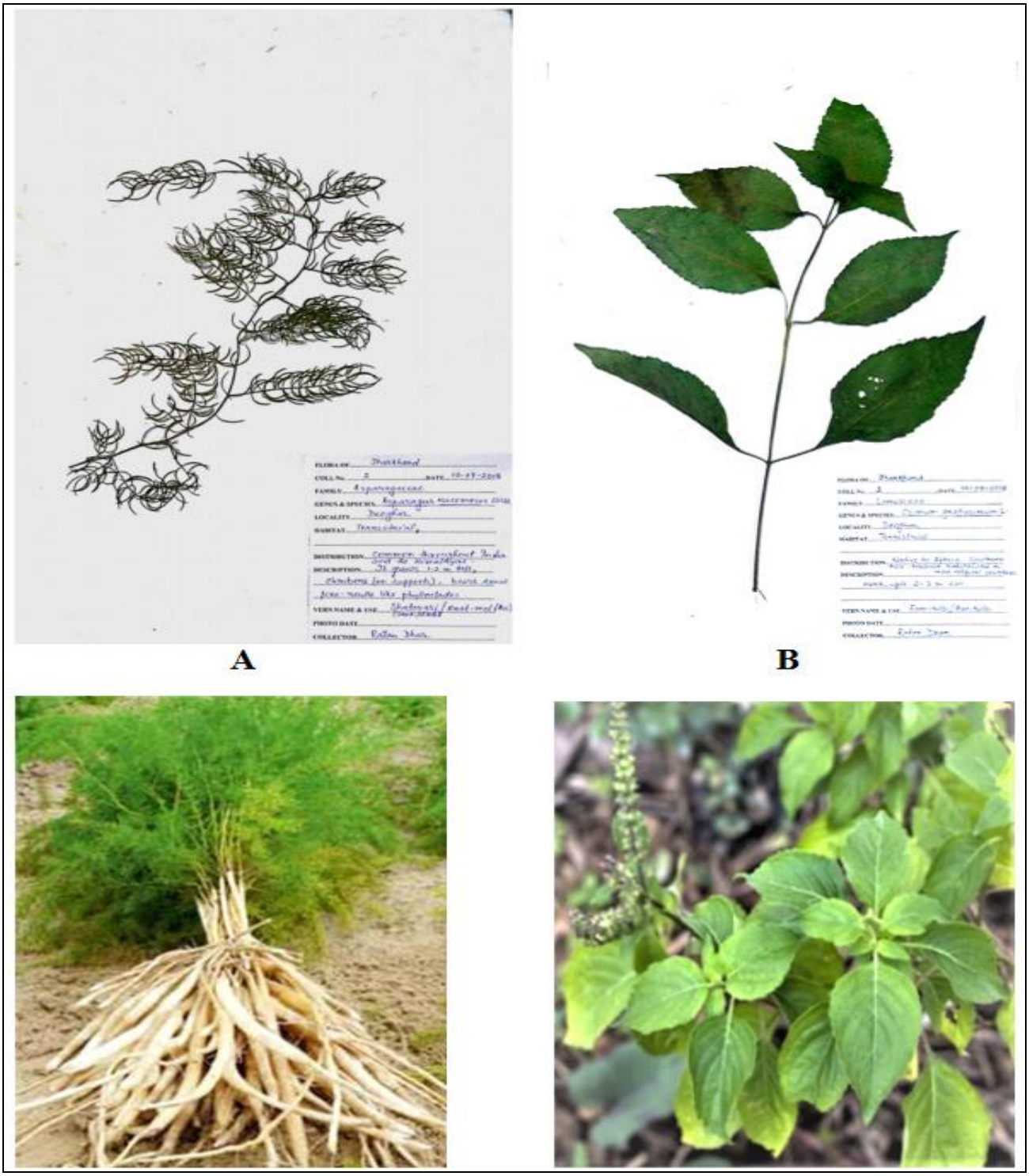

Fig 1: Herbaria of *Asparagus racemosus* (A) and *Ocimum gratissimum* (B) along with the natural plants

Antibacterial activity of *A. racemosus* and *O. gratissimum*

The antibacterial activity of *A. racemosus* and *O. gratissimum* derived from root and seed extracts prepared in ethanol and methanol is shown in Table 1-2. As a whole, the root ethanol extracts showed higher zone inhibition than the methanol extract of *A. racemosus* against all the tested bacterial strains. The highest zone inhibition was exhibited by root ethanol extract against *E. coli* (DDSCH54) with 9.33 ±0.88 mm inhibition followed by 9.33 ±0.66 mm inhibition against *K. pneumoniae* (DDSCH62) and 7.66 ± 0.88 mm against *P. mirabilis* (DDSCH72) at 20 mg/ml concentration. With increasing concentration of the extracts, the zone inhibition was found to be increased from 5.0 – 20 mg/mL. In the case of positive control (Nitrofurantoin 30 □g), the highest zone inhibition was observed against *P. mirabilis* (DDSCH72) with 13.0 ± 0.57 mm inhibition followed by *E. coli* (DDSCH54) and *K. pneumoniae* (DDSCH62) with almost equal zone of inhibition. No any inhibition was found for the negative control (DMSO) [Plate 1 - 2]. Ethanol extracts of root of *A. racemosus* showed more activity than methanol extracts. Similar findings were also recorded by Das *et al*. 2020 in *Brucea mollis* which corroborate the fact that antibacterial efficacy of ethanol extract of leaf derived callus was better than the other solvents used.

Table 1: Zone inhibition test (mm) of root extract of *Asparagus racemosus*

Extract	Concentration (mg/mL)	*E. coli* (DDSCH54)	*K. pneumoniae* (DDSCH62)	*P. mirabilis* (DDSCH72)
Ethanol	2.5	8.33 ± 0.66	7.66 ± 0.33	7.0 ± 1.15
	5.0	9.0 ± 0.57	8.66 ± 0.88	7.66 ± 0.88
	10.0	9.0 ± 0.57	9.0 ± 1.54	7.66 ± 0.66
	20.0	9.33 ±0.88	9.33 ± 0.66	7.66 ± 0.88
Methanol	2.5	7.33 ± 0.33	7.33 ± 0.33	6.66 ± 0.33
	5.0	8.0 ± 1.0	7.66 ± 0.33	6.66 ±0.88
	10.0	8.33 ± 0.66	8.0 ± 0.57	7.0 ± 0.57
	20.0	8.66 ± 0.33	8.66 ± 0.33	7.33 ± 0.66
Nitrofurantoin	30 □g	12.33 ± 0.88	12.0 ± 0.57	13.0 ± 0.57

Values are Mean ± SD of three replicates repeated thrice per experiment. mm: milimeter; □g: microgram; mg/mL; milligram per millilitre

The antibacterial activity of seed of *O. gratissimum* derived ethanol and methanol extracts were studied against *E. coli* (DDSCH54), *K. pneumoniae* (DDSCH62) and *P. mirabilis* (DDSCH72). Out of both the extracts, the maximum zone inhibition was observed for methanol extract for all the test organisms. The maximum zone of inhibition (9.4 ± 0.6 mm) was exhibited significantly by methanol extract at 20 mg/mL against *P. mirabilis* (DDSCH72) followed by 9.4 ± 0.3 mm against *K. pneumoniae* (DDSCH62) and 9.33 ± 0.2 mm inhibition against *E. coli* (DDSCH54) respectively. However, the methanol extract of the seed of *O. gratissimum* was found to be effective for all the test organisms at 5 -20 mg/mL concentrations significantly (Table 2). In the case of positive control (Nitrofurantoin 30 □g), the zone inhibition was found to be 13.0 ± 0.57 mm against *P. mirabilis* (DDSCH72), 12.0 ± 0.57 mm against *K. pneumoniae* (DDSCH62) and 12.33 ± 0.88 mm against *E. coli* (DDSCH54) respectively whereas no inhibition was occurred for the negative control (DMSO). It was also found that with the increase in the concentration of the extract, there was also an increase in antimicrobial activity against the tested bacterial strains which is corroborated by the findings of several other reports.

Table 2: Zone inhibition test (mm) of root extract of *Ocimum gratissimum*

Extract	Concentration (mg/mL)	*E. coli* (DDSCH54)	*K. pneumoniae* (DDSCH62)	*P. mirabilis* (DDSCH72)
Ethanol	2.5	6.2 ± 0.11	6.0 ± 0.44	6.0 ± 0.15
	5.0	7.33 ± 0.55	7.66 ± 0.66	7.6 ± 0.66
	10.0	7.22 ± 0.55	7.2 ± 1.45	7.6 ± 0.33
	20.0	7.33 ±0.44	7.3 ± 0.88	7.66 ± 0.33
Methanol	2.5	8.11 ± 0.3	8.22 ± 0.3	8.4 ± 0.33
	5.0	9.22 ± 1.12	8.4 ± 0.33	8.4 ±0.66
	10.0	9.3 ± 0.6	9.11 ± 0.46	8.1 ± 0.5
	20.0	9.33 ± 0.2	9.4 ± 0.3	9.4 ± 0.6
Nitrofurantoin	30 □g	12.33 ± 0.88	12.0 ± 0.57	13.0 ± 0.57

Values are Mean ± SD of three replicates repeated thrice per experiment. mm: milimeter; □g: microgram; mg/mL; milligram per milliliter

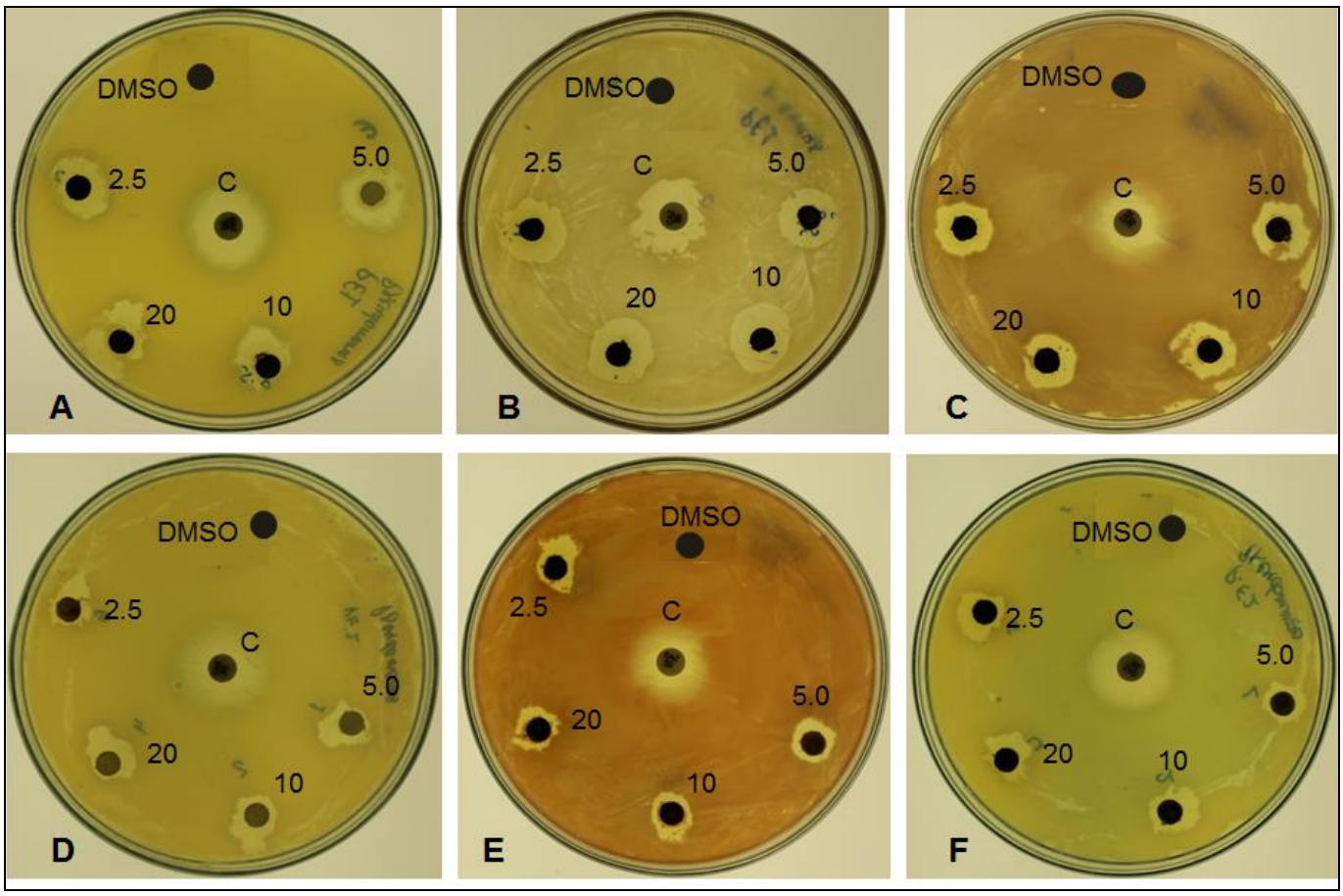

Plate 1: Antimicrobial activity of root ethanol extract of *Asparagus racemosus* against (A) *E. coli* (DDSCH54), (B) *K. pneumoniae* (DDSCH62), (C) *P. mirabilis* (DDSCH72), and seed ethanol extract of *Ocimum gratissimum* against (D) *E. coli* (DDSCH54), (E) *K. pneumoniae* (DDSCH62), (F) *P. mirabilis* (DDSCH72); label denotes the amount in mg/mL

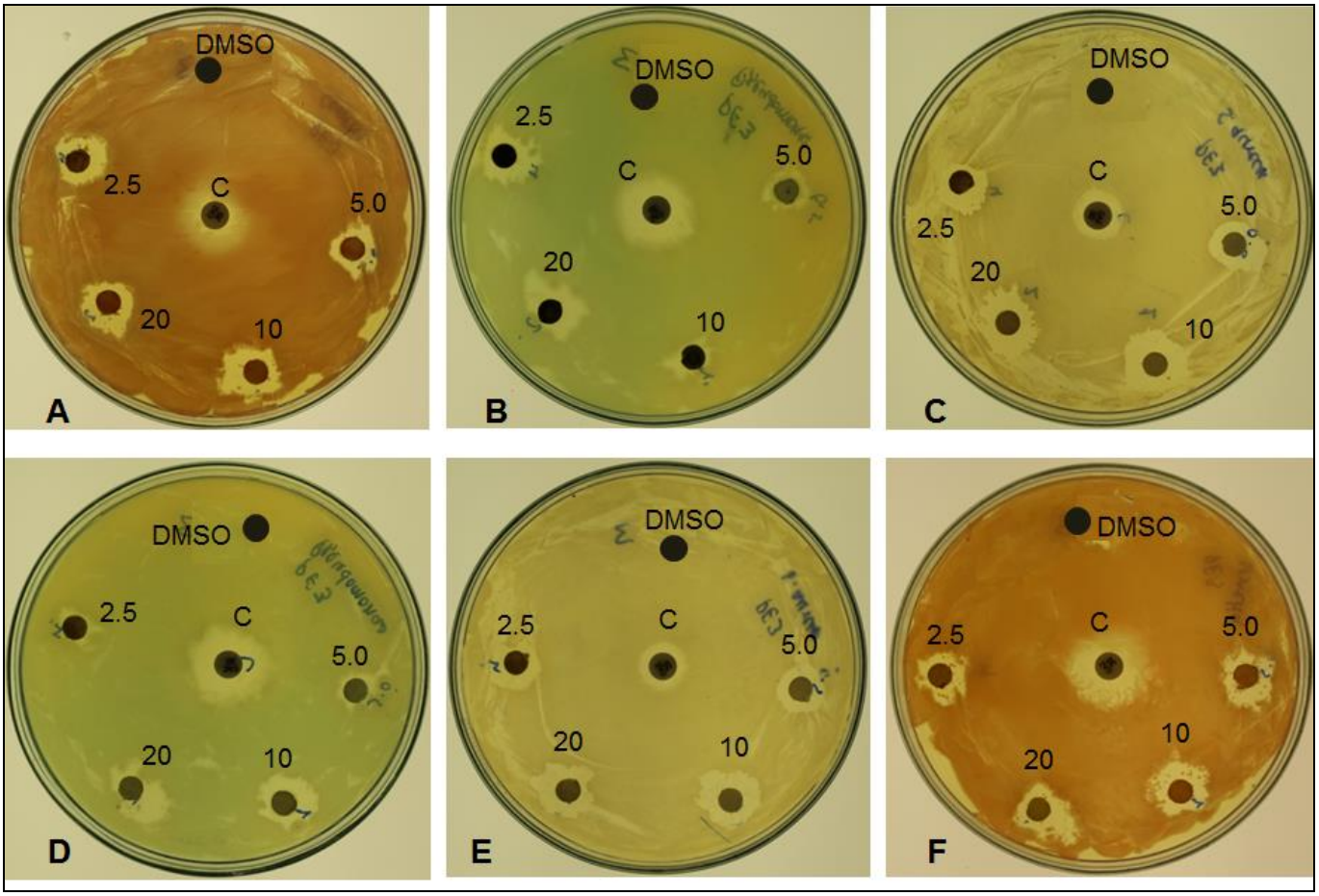

Plate 2: Antimicrobial activity of root methanol extract of *Asparagus racemosus* against (A) *E. coli* (DDSCH54), (B) *K. pneumoniae* (DDSCH62), (C) *P. mirabilis* (DDSCH72), and seed methanol extract of *Ocimum gratissimum* against (D) *E. coli* (DDSCH54), (E) *K. pneumoniae* (DDSCH62), (F) *P. mirabilis* (DDSCH72); label denotes the amount in mg/mL

Determination of minimum inhibitory concentration (MIC)

The MIC of the ethanol and methanol extracts of root and seed extracts of *Ocimum gratissimum* and *Ocimum gratissimum* against three experimental test organisms ranged from 0.078 - 0.625 mg/mL. The root ethanol extracts of *Asparagus racemosus* showed MIC at 0.156 mg/mL against *E. coli* (DDSCH54) and *K. pneumoniae* (DDSCH62) while in case of *P. mirabilis* (DDSCH72), it showed at 0.3125 mg/mL. Whereas, the seed methanol extract of *Ocimum gratissimum* showed MIC at 0.078 mg/mL against *K. pneumoniae* (DDSCH62) and *P. mirabilis* (DDSCH72). The seed ethanol extract showed MIC at 0.156 mg/mL against *K. pneumoniae* (DDSCH62) and *P. mirabilis* (DDSCH72) whereas root methanol extract showed MIC at 0.3125 mg/mL against *E. coli* (DDSCH54) and *K. pneumoniae* (DDSCH62) (Table 3).

Table 3: Minimum inhibitory concentration of the root extracts of *Asparagus racemosus* and seed extract of *Ocimum gratissimum*

Microorganisms	Minimum inhibitory concentration (mg/mL)			
	Root extract of *Asparagus racemosus*		Seed extract of *Ocimum gratissimum*	
	Ethanol	Methanol	Ethanol	Methanol
E. coli (DDSCH54)	0.156*	0.3125	0.3125	0.156*
K. pneumoniae (DDSCH62)	0.156*	0.3125	0.156	0.078*
P. mirabilis (DDSCH72)	0.3125	0.625	0.156	0.078*

*Minimum inhibitory concentration (MIC) of the root and seed extracts of *Asparagus racemosus* and *Ocimum gratissimum* against three experimental test organisms

Discussion

A number of studies on antimicrobial activity of the *Asparagus racemosus* and *Ocimum gratissimum* have been undertaken (Adebolu and Oladimeji 2005; Battu and Kumar 2010; Jesuwenu and Michael 2017; Mandal *et al* 2000; Matasyoh *et al* 2008; Onaebi *et al* 2020) [17, 18, 19, 20, 21, 22]. However, all the antimicrobial activities were studied with other bacteria and fungi with this two plants (Johnson *et al* 2011; Patel and Patel 2013; Pandey 2017) [23, 24, 25]. This is the first report to study the antibacterial activity of *Asparagus racemosus* and *Ocimum gratissimum* on few predominant UTI bacteria (Jose and Devassykutty 2016; Nakamura *et al* 1999) [26, 27]. *A. racemosus* and *O. gratissimum* are widely used in Deoghar district of Jharkhand, India for the treatment of urinary tract infection (UTI) as traditional medicines by the local communities without any scientific knowledge (Manikandan *et al* 2015) [28]. Therefore, an attempt was made in this investigation to determine the antibacterial activity against three predominant UTI bacteria from the region. The

experimental plants were selected based on traditional knowledge of the Jharkhand, India. The ethanolic extract of the leaves of *O. gratisimum* was used in traditional medicine for the treatment of several ailments including UTI by Nweze and Eze (2009) [29]. Similar kind of study was conducted by other workers for evaluation of antibacterial property from the volatile oils of the leaves of *O. gratissimum* against several Gram positive and Gram Negative bacteria along with pathogenic fungus (Matasyoh *et al* 2008) [21]. In this study, both the plant extracts showed various degrees of antimicrobial effect against the tested UTI bacteria. Both ethanolic and methanolic extracts of *A. racemosus* were found to exhibit broad-spectrum antibacterial effects which is agreement with some other reports (Patel and Patel 2013; Pandey 2017) [24, 25]. On the other hand, it was also observed the increasing antibacterial activity with the increasing concentration of the extract (Onaebi *et al* 2020; Nakamura *et al* 1999) [22, 27]. Ethanol extracts of the root of *A. racemosus* showed more activity than methanol extracts. Similar findings was also recorded by Mandal *et al* (2000) [20] in the same plant which corroborate the fact that antibacterial efficacy of ethanol extract better than the other solvents used. In contrast, in case of *O. gratissimum*, the seed methanol extract was found to be more effective than the ethanol extract which is in concordant with some other reports (Adebolu and Oladimeji 2005; Battu and Kumar 2010) [17, 18]. However, this is the first report of the antibacterial activity of seed of *O. gratissimum* used in this study based on traditional knowledge as most of the investigations were conducted with leaf extract (Jesuwenu and Michael 2017; Matasyoh *et al* 2008) [19, 21]. The differences in the inhibitory effect of both root of *A. racemosus* and seed of *O. gratissimum* derived extract against the predominant UTI bacterial strains may be due to the quantitative and qualitative variations in the bioactive compounds present in them.

Conclusion

In conclusion, the present investigation proved scientific evidence of *Asparagus racemosus* and *Ocimum gratissimum* for antibacterial activity against few predominant UTI bacteria. Therefore, further studies are necessary to identify the bioactive compounds and evaluate their antimicrobial activity against a wide range of UTI pathogens in order to confirm their efficacy as antimicrobial agent. However, emphasis should be given more to evaluate more traditional medicinal plants to identify potent antimicrobial plants against UTI. Moreover, the focus should be made in developing new effective drugs through computational knowledge.

Author contributions

PKB was involved conception and design of the study. RD carried out all the experiments and analyzed the data. PKB critically analyzed the findings. Both the authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest

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Reference

1. Joseph B, Raj SJ. Pharmacognostic and phytochemical properties of *Aloe vera* Linn: an overview. Int J Pharma Sci Rev Res,2010:4:106-110.
2. Sarma B, Tanti B. *In vitro* regeneration of plantlets from nodal explants of *Aristolochia saccata* and *Aristolochia cathcartii*. European J Biol Res,2017:7(3):191-201.
3. Tanti B, Buragohain AK, Gurung L, Kakati D. Assessment of antimicrobial and antioxidant activities of *Dendrocnide sinuata* (Blume) Chew leaves- a medicinal plant used by ethnic communities of Northeast India. Indian J Nat Prod Res,2010:1:17-21.
4. Rai PK, Jaiswal D, Singh RK, Gupta RK, Watal G. Glycemic properties of *Trichosanthes dioica* leaves. Pharma Biol,2008:46:894-899.
5. Pullaiah T. *Encyclopaedia of World Medicinal Plants.* Regency Publications, New Delhi,2006:1:360.
6. Viswanad V, Aleykutty NA, Jaykar B, Zachariah SM, Thomas L. Studies on antimicrobial and antioxidant activity of methanolic extract of *Samadera indica*. Int J Pharma Sci Rev Res,2011:11:59-64.
7. Baruah PS, Deka K, Sarma B, Das P, Borthakur SK, Tanti B. Assessment of few unexplored RET plant wealth of Assam, India. J Adv Plant Sci,2017:9(2):10-15.
8. Das P, Hasnu S, Lahkar L, Mahanta S, Borthakur SK, Tanti B. Antimicrobial activity and antioxidant properties of *Brucea mollis* Wall. ex Kurz: a medicinal plant of Northeast India. Advances in Traditional Medicine, 2020. doi: 10.1007/s13596-020-00494-y.
9. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* I.: An ethnomedicinal plant. J Adv Pharma Technol Res,2011:2:104-109.
10. Roy S, Basu S, Anitha M, Singh DK. Synergistic extraction of Nd (III) with mixture of 8-hydroxyquinoline and its derivative with di-2-ethyl hexyl phosphoric acid in different diluents. Korean J Chem Eng,2017:34(6):1740-1747.
11. Parekh J, Chanda S. *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). African J Biomed Res,2006:9:89-93.
12. Jouda MM, Elbashiti T, Masad A, Albayoumi M. The antibacterial efect of some medicinal plant extracts and their synergistic effect with antibiotics. World J Pharm Pharma Sci,2016:5:23-33.
13. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American J Clinical Pathol,1966:45:493-496.
14. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of Clinical Microbiology. 1995; Vol. 6. ASM, Washington, DC.
15. Clinical and Laboratory Standards Institute. M07–A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 8th edn. Wayne, PA: CLSI, 2009; Vol. 29 no. 2.
16. Taye B, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. Asian Pacific J Trop Biomed,2011:1:370-375.

17. Adebolu TT, Oladimeji SA. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. African Journal of Biotechnology,2005:4(7):682-684.
18. Battu GR, Kumar BM. Phytochemical and antimicrobial activity of leaf extract of *Asparagus racemosus* Willd. Pharmacognosy Journal,2010:2(12):456-463.
19. Jesuwenu AD, Michael OO. Antibacterial activity of *Ocimum gratissimum* L. against some selected human bacterial pathogens. Journal of Pharmaceutical Research International,2017:20(1):1-8.
20. Mandal SC, Nandy A, Pal M, Saha BP. Evaluation of antibacterial activity of *Asparagus racemosus* willd. Root. Phytother Res,2000:14(2):118-9.
21. Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai AWT, Mukiama TK. Antimicrobial activity of essential oils of *Ocimum Gratissimum* L. from different populations of Kenya. Afr J Tradit Complement Altern Med,2008:5(2):187-193.
22. Onaebi C, Onyeke C, Osibe D, Ugwuja F, Okoro A, Onyegirim P. Antimicrobial activity of *Ocimum gratissimum* L. and *Carica papaya* L. against postharvest pathogens of avocado pear (*Persea americana* Mill.). Journal of Plant Pathology,2020:102:319-325.
23. Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and internodal callus extracts of *Mentha arvensis* L. Asian Pacific J Tropical Med,2011:4:196-200.
24. Patel LS, Patel RS. Antimicrobial activity of *Asparagus racemosus* Wild. International Journal of Scientific and Research Publications,2013:3(3):1-3.
25. Pandey S. Antibacterial and antifungal activities of *Ocimum gratissimum* L. International Journal of Pharmacy and Pharmaceutical Sciences,2017:9(12):26-31.
26. Jose J, Devassykutty D. Evaluation of antibacterial activity of *Asparagus racemosus* in urinary tract infection. National Journal of Physiology Pharmacy and Pharmacology,2016:6(6):1. doi: 10.5455/njppp.2016.6.0617401072016.
27. Nakamura CV, Ueda-Nakamura T, Bando E, Melo AFN, Cortez DAG, Filho BPD. Antibacterial activity of *Ocimum gratissimum* L. essential oil. Mem Inst Oswaldo Cruz, Rio de Janeiro,1999:94(5):675-678.
28. Manikandan A, Rajendran R, Balachandar S, Sanumol MS, Mary Sweety M. Antimicrobial activity of *Ailanthus excelsa* Roxb. Collected from Coimbatore District, Tamil Nadu, India. World J Pharm Pharma Sci,2015:4:697-704.
29. Nweze EI, E Eze EE. Justification for the use of *Ocimum gratissimum* L in herbal medicine and its interaction with disc antibiotics. BMC Complementary and Alternative Medicine,2009:9:37. doi:10.1186/1472-6882-9-37.