

Formulation of Herbal Tea and in Vitro Evaluation of Antibacterial Activity Against Drug-Resistant Uropathogens

Kesarwani Akanksha¹, Shraddha Tripathi², Mishra Neha^{1*}, Prasad Ranu¹, Mishra Neetu²

¹Department of Food Nutrition and Public Health, SHUATS, Prayagraj – 211007.

²Department of Family and Community Sciences, University of Allahabad, Prayagraj-211011

Abstract

The present study aims to formulate herbal tea and examine antioxidant and antimicrobial activity against uropathogens. Total phenolics content and radical scavenging activity of herbs (Hibiscus, Lemongrass, and Tinospora) and optimized tea were determined by the Folin–Ciocalteu method and percent of DPPH inhibition, respectively. The antibacterial activity of selected herbs and optimized tea were tested against three clinical isolates, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the modified agar disc diffusion method. The highest phenolic content and radical scavenging activity were observed in lemongrass, followed by hibiscus flower and tinospora stem. Although, the highest antibacterial activity was recorded by hibiscus flower against all tested pathogens, followed by lemongrass and tinospora. The sensory score of different treatments of herbal tea was analyzed by the Hedonic scale and treatment T₂ (55:20:25) is found to be best followed by T₁, T₀, T₃. The radical scavenging activity and antibacterial activity of herbal tea were found to be higher than in green tea. The herbal tea also exhibit significant antibacterial activity against common uropathogens *E.coli* (0.73mm), *K.pneumoniae*(0.5 mm) and *P.aeruginosa* (0.4 cm). Therefore, the prepared herbal tea could be recommended for the prevention and treatment of urinary tract infection.

Keywords: Antioxidant, Antibacterial, Herbal Tea, Hibiscus, Lemongrass and Tinospora

*Corresponding author Email: neha.alladuniv@gmail.com

1. Introduction

Urinary Tract infection is the most widely distributed health issue among both communities and hospital settings. It is a most common infection which occurs mainly in women, and approximately 60% of women populations in the world have experienced the problem of UTI once in a lifetime, with a 25% chance of recurrence within 6 months and 46% over a 12-month period, in spite of antibiotic intervention (Alós et al; 2005). UTIs if they remain untreated, can result in serious health issues, e.g., Kidney damage and renal failure. UTIs (Urinary tract infections) are contagious infections that affect both upper and lower urinary regions, including the kidney, urinary bladder, urethra, and ureter. It is characterized by inflammation of the bladder, dysuria, recurrence, and painful urination, although it can persist with serious medical consequences such as kidney stones and acute and chronic renal failure. It was also found that prevalence of uncomplicated UTIs were higher in women than men especially during the years of maximum sexual activity, usually between the ages of 18 and 39 because the women's urethra are less effective at preventing bacterial entry (Alós et al; 2005). It has found that in spite of antibiotic intervention, 60% of women populations in world have experienced the problem of UTI once in a lifetime with 27% chance of recurrence within 6 months and 2.7% over a 12-month period (Medina and Castillo-Pino 2019). The most common methods practiced for the treatment of urinary tract infection include antibiotics and good personal hygiene. Reduction of susceptibility and raising the multidrug-resistant strain raised a need for alternative therapy.

Medicinal plants have been used to treat and prevent different types of the infectious disease since prehistoric times (Mishra et al., 2013). Nearly 60 to 90% of the total population worldwide uses plant-based medication. They are reservoirs of different types of bioactive compounds such as tannins, flavonoids, and alkaloids, which also possess antibacterial properties. While in most cases, the action mechanism and efficacy of herbal extracts still need scientific validation, which facilitates important host responses.

Hibiscus rosa-Sinensis (H. Rosa- Sinensis) is a member of the Malvaceae family. It is an evergreen woody shrub commonly cultivated in tropical and sub-tropical regions. Hibiscus has an attractive and colorful flower widely used as ornamentals. The flowers of the hibiscus rosa- Sinensis have been utilized in traditional medicine as anti-inflammatory, antifungal, antipyretic, and antihelminthic agents (Wu et al., 2005). Previous studies have shown that the Hibiscus plant is rich in several biologically active compounds, includes phenolics, flavonoids and Quercetin and has antioxidant, antimicrobial, anti-diabetic, hypocholesterolemic, and anti-cancer properties. Studies have shown that petals of Hibiscus have antibacterial activity and are used as an herbal drink worldwide.

Lemongrass, scientifically known as *Cymbopogon citratus* belongs to the Gramineae family. It is a tall perennial grass commonly cultivated in tropical and subtropical habitats (Cheel et al; 2005). They are active constituents reservoirs, including tannins, alkaloids, flavonoids, and some essential oil. Secondary active metabolites possess numerous therapeutic effects, includes posses anti stress, antioxidants, and antimicrobial properties. Citral, a cyclic monoterpene is a key constituent of biologically active compounds responsible for the characterized distinct lemon-like odor. Previous studies reported the antimicrobial activities of citral against pathogenic bacteria and fungi (Shi et al;2017).

Tinospora cordifolia (Giloy) is a climbing shrub that belongs to the family *Menispermaceae*. Different parts of the plant, including stem root, and leaves, have been used in the treatment of human ailments in the traditional system of medicine, especially in Ayurveda. It is also called as Amrita or Guduchi. They are rich in several active constituents, including steroids, alkaloids, glycosides, polysaccharides, aliphatic and phenolic compounds, saponins, flavonoids phytosterols (Singh et al; 2003). These compounds account for antimicrobial, anti inflammatory, immunostimulant antioxidant activity, anti-diabetic and anti-cancer activity (Sohan and Shyamasree 2012). Previous studies have documented the antioxidant and antimicrobial activity of *Tinospora* against broad spectrum bacteria and fungi species. Traditionally, the stem of *Tinospora* was used to treat dyspepsia, fever and urinary disease. Because of their great antibacterial activity, these medicinal plants could

be used as a tea or infusion to prevent or treat urinary tract infection. Therefore, the present work aims to formulate herbal tea and examine its antioxidant and antimicrobial activity against uropathogens.

2. Materials and Methods:

2.1 Procurement of Raw Materials:

The best quality licorice, ginger and honey were purchased from the local market of Prayagraj, Uttar Pradesh. Hibiscus flower, Tinospora stem, Lemongrass and Tulsi for the experiment were collected from the Department of Horticulture, SHUATS, Naini, Prayagraj, Uttar Pradesh.

2.2 Collection of Microorganisms:

Three clinical isolates (*Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* belonging to different bacterial species were collected from the Department of Microbiology and Fermentation Technology, SHUATS, Naini, Prayagraj, Uttar Pradesh.

1.3 Preparation of sample:

All plant materials were carefully inspected and all foreign materials were removed. The samples of the herb were rinsed in tap water. Lemongrass, Tinospora stem and Hibiscus flower spread thinly on paper and then shade dried for three-five days. After drying, the samples were milled using an electric blender. The samples were stored in glass bottles with tight lids and labeled separately. The three dried and milled herbs (lemongrass, tinospora stem and hibiscus flower) were mixed in varying proportions to obtain three different formulations. Each formulation was packed into tea bags. Each tea bag contains approximately 5g of sample. The teabags were stored in glass bottles with tight lids and labeled accordingly for sensory analysis. Commercial Green tea was used as a control.

Table 3.1- Proportion of herbs in herbal tea

Ingredients	Amount (%)
Tinospora stem	10-30
Lemongrass	50-60
Hibiscus flower	20-30

1.4 Determination of Total phenolic content:

The total phenolic compounds in each extract were assessed using Folin-Ciocalteu reagent following Jayashree's method (Jayashree and Londonkar, 2014). 0.2 ml of aliquot mixed with 5 mL of 10% folin cioalteu phenol reagent. After incubation of 5 minutes, 1ml of sodium carbonate solution (10%) was added. The absorbance was observed in a wavelength 765 nm. A standard solution of gallic acid (55-175 µmg/ml) was used to obtain a calibration curve. TPC was presented as a percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

1.5 DPPH radical scavenging activity:

The radical scavenging activity of extracts was measured by following the method described by Brand-Williams *et al*; (1995) with little modification. Different concentrations (60-240 µg/ml) of each solvent extracts were mixed to 150 ìl (0.1mM) DPPH solution and incubated at room temperature for 15 min. Methanol was used as blank, and the absorbance was measured at 517 nm using the spectrophotometer, and results were expressed as % of inhibition of the DPPH radical. A radical scavenging activity was calculated by the following formula:

$$\% \text{DPPH activity} = \frac{\text{Absorbance 517 nm of control} - \text{Absorbance 517 nm of sample}}{\text{Absorbance 517 nm of control}} \times 100$$

2.6 Antibacterial Activity Evaluation by Agar Well Diffusion Assay:

The antimicrobial activity was evaluated by agar well diffusion method (Prabuseenivasan, et al 2006). Bacterial Inoculums containing 10^6 CFU/ml was swab on the surface of sterile Mueller-Hinton agar plates using a sterile cotton swab and allow to dry for 3-5 minutes. The zone of inhibition was measured around each disc to evaluate the antibacterial activity and expressed in millimeters (mm).

2.7 Statistical Analysis:

The data was presented as mean of three replicates as Mean \pm S.D. The result were statistically analyzed by using one way Analysis of Variance (ANOVA) technique at level of significant 5% by using SSPS 16.0 version.

3. Results and Discussion:

3.1 Total Phenolic Content of Herbs

Total phenolic content present in methanolic extracts was found in a range of 17 – 42mgGAE/g. Table 1 illustrated that highest phenolic content was found in lemongrass (38.84 ± 1.14 mgGAE/g) followed by hibiscus flower (32.20 ± 2.50 mgGAE/g) and Tinospora (17.44 ± 1.12 mgGAE/g). Previous studies observed that the antioxidant capacity is highly correlated with the polyphenolic compound's contents of the plant (Singh et al; 2016). In the present study, the total phenolic content of the sample decreases in the following order: Lemongrass > Hibiscus flower > Tinospora stem. The best of our knowledge, no studies were found that have compared the phenolic compounds of selected medicinal plants. However, several studies reported significantly higher phenolic compounds in lemongrass, tinospora and hibiscus (Unuigbe et al; 2019).

3.2 DPPH Radical Scavenging Activity of Herbs:

In recent years attention has been focused on the antioxidant properties of medicinal plants that have an important role in the prevention of disease. The antioxidant activities of herbs were evaluated in terms of radical scavenging activity. The DPPH radical scavenging activity of selected herbs was expressed in term of percentage of inhibition and presented in table 1. Higher percentage inhibition indicates better scavenging activity or antioxidant potential. The obtained results shown that the antioxidant activity of all selected herbs is statistically significant different ($p < 0.05$) from each other. It varies from 29% to 35%. In a present study found that the highest radical scavenging activity was observed in lemongrass (31.2%), followed by hibiscus flower (30.9%) and Tinospora stem (29.9%). Previous studies reported the good radical scavenging activity in selected medicinal plants; higher radical scavenging activity might be attributed to the presence of high phenolic compounds in methanolic extract. Sousa et al (2021) reported the significant phenolic compound content in lemon grass and also demonstrated its DPPH free radical reducing capacity and thereby its ability for antioxidant compound. Ilaiyaraja et al (2011) also found the higher antioxidant activity in stem of Tinospora than leaves.

Table 1- Total Phenolic Content (TPC) and Dpph Activity of Herbs

Sample	mgGAE/g	DPPH %
Lemongrass	38.84 ± 1.14	31.2
Tinospora stem	17.44 ± 1.12	29.9
Hibiscus flower	32.20 ± 2.50	30.9

3.3 Antimicrobial Activity of Herbs

The antibacterial activity of methanol extracts of Hibiscus flower, Tinospora stem, and lemongrass were tested against selected uropathogens by disc diffusion method shown in table 2. The highest antibacterial activity was recorded by Hibiscus flower against all tested pathogens, followed by lemongrass and Tinospora. Hibiscus flower exhibits the highest antibacterial activity against *E. coli* (1 ± 0.17 mm), followed by K.

pneumonia (0.7± 0.04 mm), *P.aeruginosa* (0.6± 0.17 mm). Similarly, Ruban (2012) reported that the hibiscus flower exhibits the highest antibacterial activity against *E. coli*(1.1±0.16mm), followed by *K. pneumonia* (0.6± 0.04 mm), *P.aeruginosa* (0.5± 0.17mm). Tinospora stems showed the least antibacterial activity against all three selected pathogens, although the highest antibacterial activity was recorded against *E. coli* (0.3± 0.12 mm), followed by *K. pneumonia* (0.3±0.14 mm), *P.aeruginosa* (0.4± 0.17 mm) respectively. Naik (2014) reported that the Tinospora stem showed the least antibacterial activity against *E. coli* (0.2± 0.12 mm), followed by *K. pneumonia* (0.3±0.14 mm) and *P.aeruginosa*(0.4± 0.17 mm). The highest antibacterial activity of the Lemongrass stem was recorded against *P.aeruginosa* (0.7± 0.02 mm), followed by *K. pneumonia* (0.6± 0.14 mm), *E. coli* (0.5± 0.02 mm), respectively. Qian et al (2016) showed significantly antibacterial activities of fifteen different herbs against pathogenic bacteria include *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhimurium* and *Staphylococcus aureus*. In support to present study, Alshami, and Alharbi (2014) reported significant antibacterial and anti-biofilm activity of Hibiscus sabdariffa (*H. sabdariffa*) extract against uropathogenic strains. Similarly, previous studies conformed the antibacterial activity of lemon grass oil and tinospora extract against drug resistant pathogens (Naik et al 2016; Shi et al., 2017; Narayanan et al 2011).

Table 2- Antimicrobial Activity of Selected Herbs

Antimicrobial Activity of Methanolic Extracts			
Zone of inhibition (MEAN ± S.E)			
Micro-organisms	Hibiscus flower	Tinospora stem	Lemongrass
<i>E.coli</i>	1±0.17	0.3± 0.12	0.9± 0.02
<i>K.pneumoniae</i>	0.7± 0.04	0.3±0.14	0.6± 0.14
<i>P.aeruginosa.</i>	0.5± 0.17	0.4± 0.17	0.7± 0.02

3.4 Sensory Analysis of Herbal Tea

The mean of sensory scores for herbal tea were presented in figure 1. The study found the in the colour of tea becomes darker on increasing the level of plant powder. T₂ has the highest score (7.53), followed by T₁ (6.73), T₀ (6.46), and T₃ (6.13). It indicates that herbal tea incorporated with 55% Lemongrass, 20% Tinospora stem, 25% Hibiscus flower i.e. T₂ (7.53) has improved color and appearance

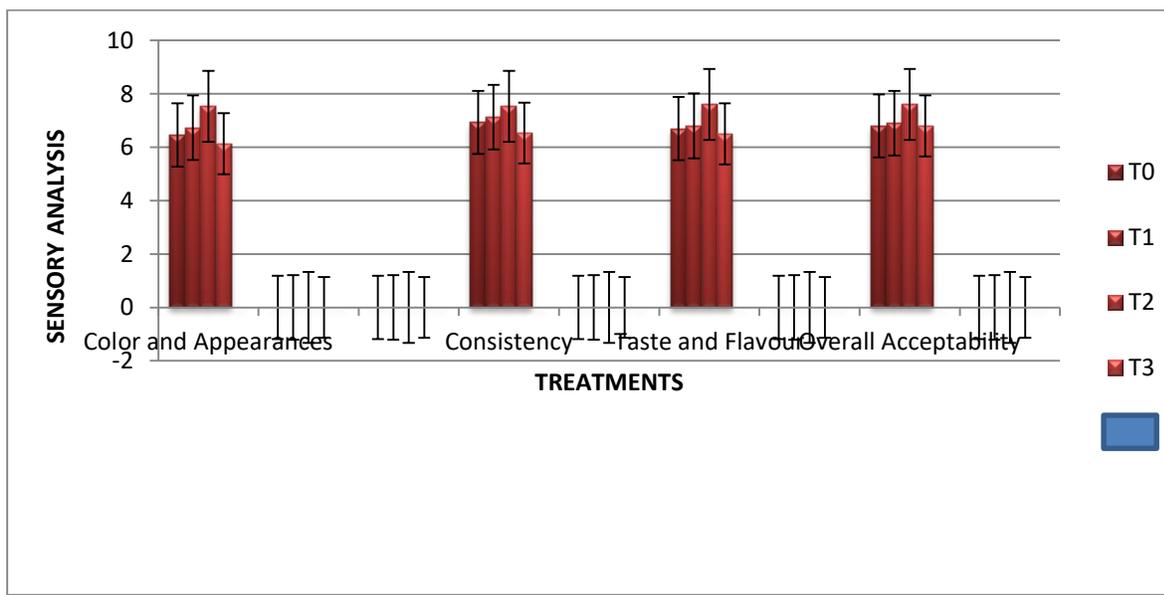


Figure 1: Average sensory scores of herbal tea

and is best in selected treatments.

The mean of sensory scores obtained for herbal tea in relation to consistency shows that T₂ has the highest score (7.53), followed by T₁ (7.13), T₀ (6.93) and T₃ (6.53). It found that consistency of tea increases with increasing the level of hibiscus powder. Figure 1 shows that the mean of sensory scores obtained for herbal tea in relation to taste and flavor, shows that T₂ has the highest score (7.6), followed by T₁ (6.8), T₀ (6.7) and T₃ (6.5). The mean of sensory scores obtained for herbal tea in relation to overall acceptability indicates that T₂ has the highest score (7.6), followed by T₁ (6.9), T₀ (6.8) and T₃ (6.8) and concluded that herbal tea incorporated with 55% Lemongrass, 20% Tinospora stem, 25% Hibiscus flower i.e. T₂ (7.6) has highest overall acceptability.

3.5 Nutritional Composition of Herbal Tea

Table 3 shows the nutritional composition of the herbal tea and found that liquorice flavored tea, T₃(60:10:30) is 2.71g have higher moisture content, followed by T₁, T₂ and T₀ is 1.91g , 1.62g , 1.64 g, respectively. Our study found that T₃ (60:10:30) has higher ash content 5.2g followed by T₁ , T₀ , T₂ is 3.23g , 2.6g , 2.57g, respectively. However, the protein content was found highest in T₂ (50:30:20) is 1.13g followed by T₀ , T₁, and T₃ is 0.5, 0.53g, 1.13g, respectively. Higher ash content observed in T₃ (55:20:25) is 5.39g have, followed by T₁,T₃ and T₀ is 4.94g, 2.98g, 3g, respectively, and Fat content was highest in T₁ (50:30:20) is 0.19g followed by T₂ , T₃ and T₀ is 0.18g ,0.17g , 0g respectively.

Table 3- Nutritional Composition of Herbal Tea

Treatments	Moisture (g)	Ash (g)	Protein (g)	Carbohydrate (g)	Fat (g)
T ₀	1.64	2.6	0.5	3	0
T ₁	1.91	3.23	0.53	4.94	0.19
T ₂	1.62	2.57	1.13	5.39	0.18
T ₃	2.71	5.2	0.8	2.98	0.174

3.6 Total Phenolic Content of Herbal Tea

In the present study, the highest phenolic content was observed in T₃(20.54 mg GAE/g) followed by T₂ (22.4 mg GAE/g) and T₁(23.68mg GAE/g). The phenolic content of best accepted herbal tea T₂ (55:20:25) is significantly higher than the phenolic content of green tea, as presented in Figure 2. It has been observed that the antioxidant capacity is highly correlated with the polyphenolic compound's contents in plants (Narayanan et al 2011; Namdev and Gupta 2015). Therefore, it might be suggested that herbal tea has higher antioxidant activity than green tea, although further study is needed.

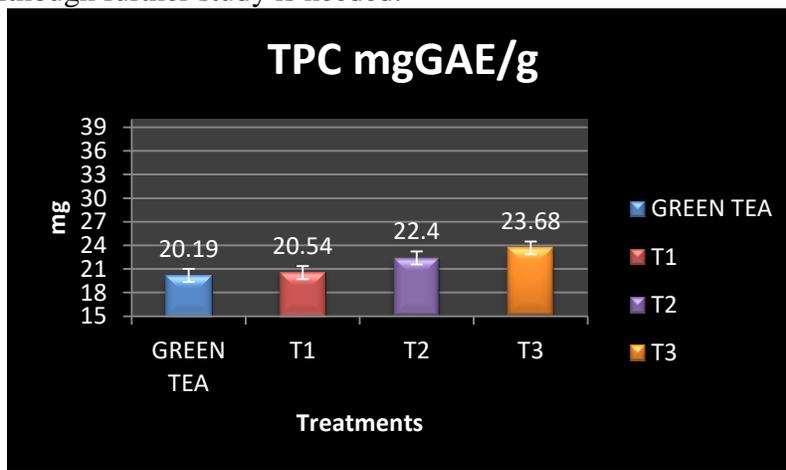


Figure 2: Total phenolic content of herbal tea

3.7 DPPH Free Radical Scavenging Activity (%) Of Herbal Tea:

In present study observed that the highest radical scavenging activity was observed in T₁(33.25%), T₂ (35%),T₃(36.41%). The DPPH free radical scavenging activity (%) of optimized herbal tea T₂(55:20:25) is significantly higher than the phenolic content of green tea (31.3%) as presented in Figure 3. Similarly, Namdev and Gupta (2015) formulated the herbal tea using *Withania somnifera* stems, Cinnamon bark, *Tinospora cordifolia* stems, *Terminalia arjuna* bark and found promising antioxidant activity. Higher radical scavenging activity might be attributed to the presence of high phenolic compounds.

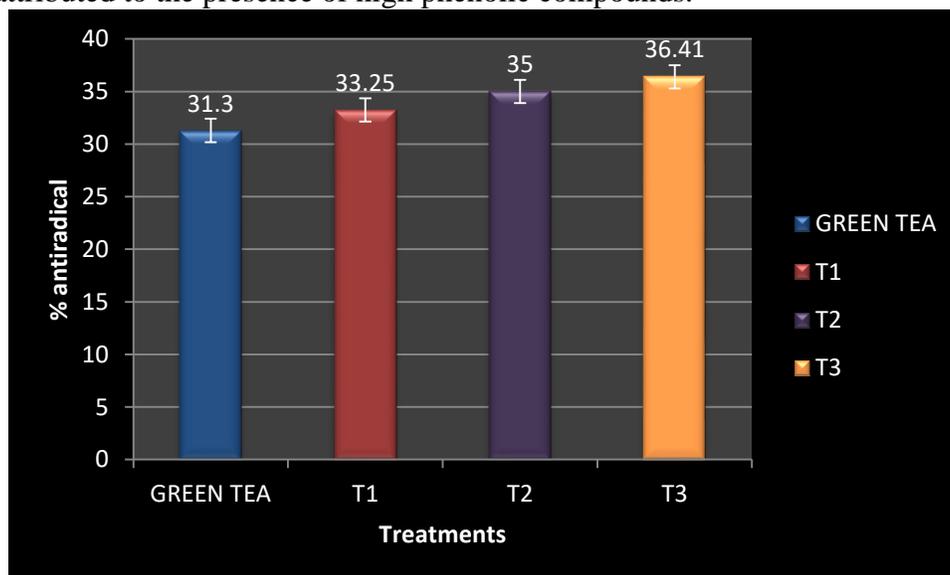


Figure 3: DPPH radical scavenging activity of herbal tea

3.8 Antibacterial Activity Of Methanolic Extracts of Herbal Tea

The highest antibacterial activity was observed T₃(0.75-0.43mm), followed by T₂(0.73-0.4mm) and T₁(0.5-0.3mm). However, the antibacterial activity of T₂ and T₃ do not differ significantly from each other, but the difference is significant with the antibacterial activity of green tea. Best treatment T₂ exhibits the highest inhibitory effect against *E.coli* (0.7±0.05 mm) followed by *K. pneumonia* (0.5± 0.03 mm), *P.aeruginosa* (0.4± 0.02 mm) as shown in table 4. Green Tea exhibits highest antibacterial activity against *E.coli* (0.6±0.04 mm), followed by *K. pneumonia* (0.4± 0.03 mm), *P.aeruginosa* (0.2± 0.04 mm). Inconsistent with a previous study, Tang (2016) reported that the highest antibacterial activity of black tea against *E.coli* (0.8±0.05 mm) followed by *K. pneumonia* (0.6± 0.03 mm), *P.aeruginosa* (0.5± 0.02 mm) respectively. The best of our knowledge, no studies were found that have the combination of these medicinal plants in herbal tea. Hacıoglu et al (2017) examined the antimicrobial activities of 31 herbal teas both alone and in combination with antibiotics or antifungals against some standard and clinical isolates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, methicillin susceptible/resistant *Staphylococcus aureus* and *Candida albicans*. Similarly, the antibacterial activity against pathogenic bacteria were documented by previous studies (Oh et al 2013; Ramamoorthy et al 2019).

Table 4- Antibacterial Activity of Methanolic Extracts of Herbal Tea

Antimicrobial Activity of Methanolic Extracts of herbal tea				
Zone of inhibition(mm) (MEAN ± S.E)				
Micro-organisms	T ₁	T ₂	T ₃	Green Tea
<i>E.coli</i>	0.5±.02	0.7±0.05	0.75±.03	0.6±0.04

<i>K.pneumoniae</i>	0.2±.01	0.5± 0.03	0.5±.05	0.4± 0.03
<i>P.aeruginosa.</i>	0.3±.02	0.4±0.02	0.43±.02	0.2±0.04

4 Conclusions

From this study, finding reveals and concludes that Tinospora stem, hibiscus flower, and lemongrass are rich in antioxidants and possess a significant antibacterial activity against most common uropathogens. The herbal tea T₂ containing 55% Lemongrass, 20% Tinospora stem, 25% Hibiscus flower (55:20:25) was highly acceptable on the basis of overall acceptability. The total phenolic content and antioxidant value of herbal tea T₂ was 22.4 mgGAE/ g and 35 % respectively. The herbal tea also exhibits significant antibacterial activity against common uropathogens *E.coli* (0.73mm), *K.pneumoniae*(0.5 mm) and *P.aeruginosa* (0.4 cm). Therefore, the prepared herbal tea could be recommended for prevention and treatment of urinary tract infection; although further studies are needed.

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