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Research article

Phytochemical analysis of *Ocimum sanctum and Swertia chirayita,* and antimicrobial properties of *Ocimum sanctum* against some bacterial strains isolated from poultry

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A B S T R A C T

Ocimum sanctum (Tulsi) and *Swertia chirayita* (Chirota) are well known source of traditional medicine. The aims of the present study are to screening the phytochemical compounds and antimicrobial sensitivity of locally available *Ocimum sanctum* and *Swertia chirayita* ethanolic extracts. Tulshi and Chirota plants were collected from local market, Chattogram. Both *O. sanctum* and *S. chirayita* were submerged in 70% ethanol for a three different time schedules like 7 days, 14 days, and 21 days, respectively. Phyto-chemical screening were done to know the presence of secondary metabolites like alkaloids, flavonoids, saponins, tannins, phenolic compounds, glycosides, carbohydrates, reducing sugar, protein and amino acid, acidic compound, phytosterol, steroids and terpenes. Three different concentrations of 0.2 mg/µL, 0.3 mg/µL, and 0.4 mg/µL ethanolic plant extracts of were treated against *E. coli*, *Salmonella* spp isolated from poultry and *Staphylococcus aureus* (ATTC -29213) as commercial strain. Of the studied phytochemicals, in *Ocimum sanctum* extracts, tannins and reducing sugar (day 7), tannins and acidic compound (day 14), and acidic compound (day 21) were absent. While in *S. chirayita*, saponins, phenolic compounds, glycosides, proteins, and amino acids, acidic compounds (day 7), acidic compounds (day 14), and phenolic and acidic compounds (day 21) of extracts were absent. Anti-microbial sensitivity of *O. sanctum* extracts at different concentrations was assessed while commercial antimicrobials disc (ciprofloxacin, amoxicillin, enrofloxacin, colistin, and sulfamethoxazole) were also assessed. *O. sanctum* extracts showed the highest zone of inhibition at 0.2 mg/µl of 14 days against *E. coli* (7-9 mm), *Salmonella* spp. (17-19 mm) Moreover, *Staphylococcus aureus* (14-15 mm) is similar to the intermediary zone sensitivity of ciprofloxacin, sulfamethoxazole, and amoxicillin. Within commercial antibiotics, ciprofloxacin, amoxicillin, enrofloxacin, and sulfamethoxazole were 100% sensitive, whereas colistin was 100% resistant. *E. coli* showed the highest percentage of sensitivity to antibiotics among other bacterial species included in this study, whereas *Salmonella* spp. had the lowest rate. In conclusion, we may say that 0.2 mg/ µL of *O. sanctum* extracts showed an intermediary zone of sensitivity against *E. coli*, *Salmonella* spp., and *Staphylococcus aureus.*

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1. INTRODUCTION

Application of traditional medicinal plants for treating diverse health conditions has been observed throughout numerous global civilizations for an extensive period. These medicinal plants provide a large store of bioactive compounds that hold promise for developing potent medicinal drugs (Wang et al., 2021). Bioactive chemicals refer to elements present in foods at modest levels that offer health advantages beyond the fundamental nutritional value of the product (Kitts, 1994). Several bioactive substances that have been identified include carotenoids, flavonoids, carnitine, choline, coenzyme Q, dithiolthiones, phytosterols, phytoestrogens, glucosinolates, polyphenols, and taurine. Traditional medicine serves as the primary source of healthcare for almost 80% of the world's population (Kunwar and Adhikari, 2005). The conventional medicine system is beneficial. Despite the advantages offered by current commercial ingredients, plants continue to play a vital role in healthcare due to the extensive recognition of therapeutic plants derived from indigenous pharmaceuticals (Adebolu, 2005).

Eugenol, carvacrol, methyl eugenol, and caryophyllene are the primary chemical components of this plant. Reduced stress is one characteristic that makes the Tulsi plant such an effective medicinal herb. Tulsi is rich in antioxidants and essential oils that are very efficient at lessening the adverse effects of stress on the body. The type of chemicals found in the Ocimum sanctum extract can be determined using phytochemical analysis (Gupta, 2002; Khanna, 2003).

Swertia chirayita, a medicinal herb indigenous to the temperate Himalayas, has been used in traditional medicine to treat various ailments such as liver disorders, malaria, and diabetes ((Kumar and Van Staden, 2016)). Chemical analysis of *Swertia chirayita* has revealed various chemical constituents, including xanthones, seco-iridoids, terpenoids, alkaloids, and flavonoids ((Swati et al., 2023a)). The therapeutic properties of *Swertia chirayita*, a medicinal plant frequently employed in traditional medicine, have been the subject of investigation regarding its potential in wound healing and skin regeneration (Velnar et al., 2009). The wound healing process encompasses a variety of cellular and molecular pathways, which a range of substances, such as growth factors, cytokines, and components of the extracellular matrix, can modulate (Gantwerker and Hom, 2012; Velnar et al., 2009). Ayurvedic health tonics, vitamins, anti-diabetic and anti-cancer medications, liver tonics, skin lotions, soaps, and hair oils all contain it as a critical component. This species was initially included in the Edinburgh Pharmacopoeia in 1839, and it is said to be used as an infusion or tincture in the British and American Pharmacopoeias. Locals in Nepal utilize this plant as an infusion made by grinding it and steeping it in water for an extended period. *Swertia chirayita* has also only lately been attempted to cultivate, with mixed results. Nature has supplied a vast array of treatments to treat human sickness. The primary healthcare needs of almost 80% of the world's population are entirely or partially met by conventional medicine. Plants have medical benefits because of their phytochemicals, which have actual physiological effects on people. Phytochemicals are substances found in plants utilized as food and medication to resist disease and uphold human health. The issue of antimicrobial resistance is a matter of global concern that presents a substantial risk to the well-being of the general population (De Oliveira et al., 2020). Multidrug-resistant organisms (MDROs) have emerged as a significant contributor to the issue of antibiotic resistance, hence exacerbating the global concern surrounding this matter.

Due to the widespread growth of microbial pathogen resistance against currently available antimicrobials, the medical community desperately needs to discover antibiotics. Traditional herbs, however, are a superior source of antibacterial medications. Antimicrobial resistance is a serious hazard to public health, particularly in poor nations. It has been established that a significant portion of the issue is caused by the existence of transferable plasmids that encode multidrug resistance and their spread among various entero bacterial species (Blake et al., 2003). Through the food chain, resistant bacteria are spread from food animals to humans. Pathogenic and commensal bacteria in the gut can exchange mobile genetic

elements influencing resistance after consuming contaminated food. Recent epidemiological investigations have shown that resistant E. coli infections in humans are rising. *Salmonella* species and *E. coli*. Invasive disease risk, hospitalization risk, and excess mortality are linked to an extended illness (Molbak, 2004).

2. MATERIALS AND METHODS

Preparation of plant extracts

Mature and disease-free *Ocimum sanctum* (Tulsi) and *Swertia chirayita* (Chirota) were collected from the local market of Chattogram. Collected plant leaves were thoroughly cleaned by washing and discarding all the unwanted materials. The aged leaves were discarded. The leaves were air-dried at room temperature for ten days to remove moisture. The dried leaves were milled to fine powder with a blender (Bajaj®, Model GX3-410176, India), and then a sieve was used to obtain fine powder dust and preserved them in an air tight plastic bag in the dark until used. 50 g of tulsi and chirota were taken into a bottle with 500 ml 80% ethanol. Then, the mixture was stirred for 10 minutes and left for three-time schedules, such as 7 days, 14 days and 21 days. The mixture was shaken and kept in a cool, dark place every day overnight. After 7 days, one batches of tulsi and chirota was taken for filtration. The mixture of tulsi and chirota was filtered separately through Whatman's Filter Paper No.1 (Tambekarand Dahikar, 2010). The filtrate was then taken into a volumetric flask fitted with the rotary evaporator (Shanghai Bio-chemicals, Model BC-4201, China). The temperature was raised to 50 °C, the alcohol was evaporated, and the extract was set in the volumetric flask. The extract was then collected in a 50mL falcon tube and stored at four °C for further study.

Phytochemical activity test

Phytochemicals present in the leaf extracts were screened as per standard protocols for phytochemical constituents such as alkaloids, glycosides, tannins, saponins, phenols, flavonoids, Steroids and Terpenes, Phenolic Compounds, Glycosides, etc. Phytochemical constituents of both *Ocimum sanctum* and *Swertia chirality* extracts were detected according to the following methods described by Sofowora (1993).

Detection of alkaloids

Mayer's Test*:* The Mayer's reagent was prepared by dissolving 1.36 g of mercuric chloride in 60 ml of distilled water. It added a solution of 5 g of potassium iodide in 20 ml distilled water. Both the solutions were mixed and diluted to 100 ml with distilled water. A few drops of the above reagent were added to a small quantity of the test residue. The formation of a cream-colored precipitate showed the presence of alkaloids (Sofowora, 1993).

Wagner's test*:* 1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water, and the solution was diluted to 100 ml with water. When a few drops of this reagent were added to the test residue, a reddish-brown precipitate formed, indicating the presence of alkaloids (Sofowora, 1993).

Detection of flavonoids

Alkaline Reagent Test*:* Extracts were treated with 4-5 drops of sodium hydroxide solution. The formation of an intense yellow color, which becomes colorless with the addition of dilute acid, indicates the presence of flavonoids (Sofowora, 1993).

Detection of saponins

Foam test*:* About 2g of the plant extract was mixed with 10ml of distilled water and shaken vigorously for a stable, persistent froth. The appearance of froth indicates the presence of saponins (Sofowora, 1993).

Detection of tannins

Lead acetate test*:* 2ml of plant extract was combined with 2ml of distilled water. 0.01g lead acetate was added to this combined solution and shaken well. The development of white turbidity and precipitate indicates the presence of tannins (Sofowora, 1993).

Detection of phenolic compound

Ferric chloride test*:* About 2ml plant extract was taken to water and warmed at 45-50^{0}C. Then, 2 ml of 0.3% $FeCl_3$ were added. The

formation of green or blue color indicates the presence of phenols (Sofowora, 1993).

Detection of glycosides

Cardiac glycosides (Killer-Killani test): 1 ml of glacial acetic acid containing traces of ferric chloride and one ml of concentrated sulphuric acid were added to the test residue and observed for the formation of reddish brown colour at the junction of two layers. The upper layer turned bluish-green with the glycosides' presence (Sofowora, 1993).

Anthraquinone glycosides (Borntrager's test): 1 ml benzene was added to the test residue, and 0.5 ml of dilute ammonia solution, no pink to red colour was formed due to the absence of glycoside (Sofowora, 1993).

Detection of carbohydrates

Molisch's test: The Molish's reagent was prepared by dissolving 10 g of α-naphthol in 100 ml of 95% alcohol. A few mg of the test residue was mixed with 2 drops of Molisch's reagent. To this solution, 1 ml of concentrated sulphuric acid was added from the inclined test tube's side. No reddish violet ring at the junction of the two layers appeared in the presence of sugars (Sofowora, 1993).

Detection of reducing sugar

Benedict's test*:* 0.5 ml of aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes, and allowed to cool spontaneously. No red colour precipitate of cuprous oxide was formed in the absence of a reducing sugar (Sofowora, 1993).

Fehling's solution test*:* A little of the test residue was dissolved in water, and a few ml of Fehling's solution was added to it. This mixture was then warmed. If a red precipitate of cuprous oxide was obtained, reducing sugars were present (Sofowora, 1993).

Detection of protein and amino acid

Xanthoproteic test*:* To the 2 ml of extract, 0.5 ml of concentrated nitric acid was added by the side of the test tube. The absence of yellow color showed the absence of proteins and amino acids (Sofowora, 1993).

Detection of acidic compound

Sodium bicarbonate test*:* To the alcoholic extract sodium bicarbonate solution was added and observed for the formation of effervescence (Sofowora, 1993).

Detection of phytosterol

Liebermann-burchard's test*:* 2 mg of the extract was dissolved in 2ml of acetic acid anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the side test tube. A brown ring formation at the junction confirmed the test for phytosterols (Sofowora, 1993).

Detection of terpenoids

Salkowski test*:* Approximately 2mg of dry extracts was shaken with 1ml of chloroform, and a few drops of concentrated sulfuric acid were added along the side of a test tube. A red-brown color formed at the interface indicated the test as positive for triterpenoids (Sofowora, 1993).

Antimicrobial discs

Each of the discs was cut from Whatman's No.1 filter paper with an approximate diameter of 6 mm using a puncher. The prepared filter paper disc was sterilized by autoclaving at 121°C for15 minutes and impregnated with 30µl extract of the experimental plant. The plant extract was dissolved in 0.2% DMSO (Di-Methyl-Sulp-Oxide) to prepare three different concentrations such as 0.2 mg/µL, 0.3 mg/µL and 0.4 mg/µL. Total six types of doses were prepared, including 14 days and 21 days batch of these 3 concentrations.

A total of 28 cultures of *E. coli* (n=14) and *salmonella* spp. (n=14) were used for sensitivity test against plant extracts and commercial antimicrobial such as ciprofloxacin, amoxicillin, enrofloxacin, colistin and colistin. The colonies were dissolved in PBS (Phosphate Buffer Saline to obtain the optimum turbidity against the 0.5 McFarland standard concentrations. After measuring the equivalence of the turbidity, the bacterial culture was ready for sensitivity test.

Isolation of bacterial samples

A total of 14 *Salmonella* spp. Isolates were collected from commercial broiler (n=12), layer (n=1) and Sonali chicken (n=1) and 14 *E. coli* isolates were collected from commercial broiler (n=10), layer (n=2), Sonali (n=1) and duck (n=1), respectively. For this study, liver samples were collected from the birds aseptically. After proper searing, the swabs were taken from the liver samples to isolate *E. coli* and *Salmonella* spp. To achieve that, each swab sample was streaked in nutrient agar and further cultured in the specific media following the procedure described by Collins and Lyne (1976). For E.*coli*, the MacConkey agar was used, and for *Salmonella* spp, XLD agar was used. A large pink colony in MacConkey agar was indicative of *E. coli*, whereas a black centered red colony in XLD agar was indicative *of Salmonella* spp. From each petri plate, 3 or 4 pure distinct colonies were taken as pure isolates, incubated overnight in nutrient broth, and kept in the freezer (-20°C) for further studies.

Antimicrobial disc preparation

To investigate the antimicrobial property of plant extracts, the 6mm discs were prepared from Whatman's No.1, kept in a screw-capped bottle, and autoclaved at 121°C for 15 minutes. The plant extracts was dissolved in 2% DMSO (Di-Methyl-Sulph-Oxide) to prepare three different concentrations; 0.2mg/µL, 0.3mg/µL, and 0.4mg/µL. Each disc was then soaked by 30µl extracts solutions (Okigbo and Mmeka, 2006). Total six types of doses were prepared including 14 days and 21 days batch of these 3 concentrations.

Culture and sensitivity test

A total of 10 cultures of *E. coli* (n=5) and *Salmonella* spp. (n=5) were used for culture and sensitivity tests against Ciprofloxacin, Enrofloxacin, Colistin sulfate, Tetracycline and Trimethoprim, and the plant extracts. The isolates were further grown in blood agar media, and then, a pure colony was isolated. The colonies were dissolved in PBS (Phosphate Buffer Saline) to obtain the optimum turbidity against the 0.5 McFarland standard concentrations (99.5 mL of 1% H2SO4added with 0.5 mL of 1.175% BaCl2). After of assessing the equivalence the turbidity, the bacterial culture was seeded for bioassay.

Bioassay

For the culture and sensitivity test, Mueller Hinton agar (Himedia, India) medium was used for the disk diffusion method (Bauer et al., 1966). The agar was prepared and autoclaved to kill any contaminating organisms and settled in petri-dishes. Then, bacterial isolates having proper turbidity were streaked (5 for *E. coli* and 5 for *Salmonella* spp.). For the bioassay, five standard commercial anti-microbial discs (Ciprofloxacin, Enrofloxacin, Colistin Sulphate, Tetracycline, and Trimethoprim from Himedia, India) were taken. On the other hand, six different discs were prepared from three different concentrations (1mg/µL, 0.5mg/µL, and 0.25mg/µL) with two different doses (10µL and 20 µL). For negative control, discs soaked with 20µL of 2% DMSO were used. In total, 12 discs were used for each bacterial isolate. To facilitate this, each isolate was grown in three plates having four antimicrobials or extract-soaked discs. The agar media was then incubated at 37°C overnight, and the zone of inhibition (mm) for each antimicrobial and plant extract-soaked disc was measured.

Interpretation and statistical analysis

From the petri-dishes, the zone of inhibitions was measured using scale. For the standard antimicrobials, the CLSI, 2007 guideline was followed. For the plant extracts, the zone of inhibitions was also recorded. All the data were entered into MS-Excel-2013, and descriptive statistical analysis (%, minimum, maximum, and mean ±SD) were performed.

Sample preparation

The collected plant leaves were properly cleaned by washing away any waste, and the older leaves were thrown away. To get rid of moisture, the leaves were air-dried at room temperature for ten days. The dried leaves were ground into a fine powder using a blender, then sieved to create fine dust, which was then stored in an airtight plastic bag until needed in the dark.

Preparation of extract

500 ml of 80% ethanol was added to a bottle with 50 g of Tulsi and Chirota. After stirring for 10 minutes, the mixture was left for three intervals of seven, fourteen, and twenty-one day. Every day, the mixture was shaken and stored in a cool, dark place. One batch of Tulsi and Chirota was taken for filtering after 7 days. The entire Tulsi and Chirota mixture was individually filtered using Whatman's Filter Paper No. 1. The filtrates were then transferred to a flask with a circular bottom in the rotator vacuum evaporator. The extracts were kept after evaporation in a Petri dish and kept in a freezer (4°C).

Phytochemical screening

Phytochemical examinations were carried out for the extracts of Tulsi, Chirota, according to the standard methods (Sofowora, 1993). The following procedure analyzed the extract to test for alkaloids, glycosides, tannins, saponins, phenols, flavonoids, phenolic compounds, steroids, and terpenes. Then, all observations were noted.

Disc preparation and antimicrobial sensitivity test

The extract was measured and combined with ethanol at a known concentration. Whatmann No. 1 filter paper was used in the meanwhile to create discs. Different extract concentrations (50,100,150, and 200mg) were put into discs. The standard disc diffusion method by Bauer et al.,, 1966 was followed to evaluate the microbial activity of poultry isolates such as Escherichia coli, *Salmonella* spp. and *Staphylococcus aureus*.

3. RESULTS

The identification of secondary metabolites was accomplished using the qualitative phyto-chemical examination. Tulsi and Chirota plant ethanolic extracts had their in vitro antibacterial properties tested. Agar disc diffusion was used to conduct the antibacterial assay for solvent extract.

Tables 1 and 2 overview the phytochemical analysis of three batches of ethanolic extracts from *Ocimum sanctum* (tulsi) and *Swertia chirayita* (chirota) leaves. Ethanolic extracts include a variety of secondary metabolites, like alkaloids, carbohydrates, tannins, flavonoids, saponins, phytosterols, glycosides, and terpenoids. Eugenol, methyleugenol, and caryophyllene are among the chemicals found in *Ocimum sanctum* leaves that are water-soluble phenolic compounds and may have immune stimulatory properties. According to Bairwa et al. (2012), saponins have anti-hyperlipidemic, hypotensive, and cardio-depressant effects. Alkaloids, steroids, flavonoids, tannins, phenols, and other aromatic compounds are only a few examples of the phytochemical components that plants produce to protect themselves against herbivores, insects, and other predators (Bonjar et al., 2004). In heart failure, glycosides can function as cardio stimulants (Sood et al., 2005). According to Asquith et al. (1986), tannins have antidiarrheal and hemostasis effects. Alcoholic extract improves acetylcholine esterase inhibition and step-down latency and is therefore utilized to treat cognitive problems.

Antimicrobial results

There were 28 bacterial isolates of *E. coli* (n=14) and *Salmonella* spp. (n=14) were used for sensitivity test against plant extracts and commercial antimicrobial such as ciprofloxacin, amoxicillin, enrofloxacin, colistin and colistin. Of the tested samples, 100% *E. coli isolates* was not sensitive to 0.3 mg/µL and 0.4 mg/µL concentrations of *O. sanctum* whereas in 0.2 mg/µL concentrations of the *O. sanctum* extracts showed sensitivity to 20% E. *coli* isolates of broiler chicken. 17% of *Salmonella* spp. isolates of broiler were sensitive to O. sanctum extracts of all stated concentrations while *Salmonella* spp. isolates of Layer, Sonali and duck were not sensitive to O. sanctum extracts. Of the total tested samples, 2 sample of both *E. coli* and *Salmonella* spp *each* show sensitivity to *Ocimum sanctum* extracts.

Table 3 shows that against all three bacterial isolates, the extracts from 14 days (S1) and 21 days (S2) had the maximum zone of inhibition at 0.2 mg/L (20%) concentration. However, neither 0.3 mg/L (30%) nor 0.4 mg/L (40%) of the concentration displayed any zone of inhibition against *E. coli* isolates. Other concentrations at various doses revealed zones of inhibition with various diameters.

Table 1. Presence of phytochemicals in three different extracts of *Ocimum sanctum* (Tulsi)

Phytochemicas	**Test**	**7-Days extract**	**14-Days extract**	**21-Days extract**
Alkaloids	Mayer's (Sofowora, 1993)	+	+	+
Flavonoids	Alkaline Reagent Test (Sofowora, 1993)	+	+	+
Saponins	Frothing (Sofowora, 1993)	+	+	+
Tannins	Lead acetate Test (Sofowora, 1993)	-	-	+
Phenolic compound	Ferric Chloride Test (Sofowora, 1993)	+	+	+
Glycosides	Killer killani Test (Sofowora, 1993) Borntranger's Test ((Sofowora, 1993)	+	+	+
Carbohydrates	Molish's Test ((Sofowora, 1993)	+	+	+
Reducing sugar	Benedict's Test (Sofowora, 1993) Feheling's Solution Test (Sofowora, 1993)	-	+	+
Protein and amino acid	Xantoproteic Test (Sofowora, 1993)	+	+	+
Acidic compound	Sodium Bicarbonet Test (Sofowora, 1993)	+	-	-
Phytosterol	Libermann-Burchad's Test (Sofowora, 1993)	+	+	+
Steroids and terpenes	Salkowski Test (Sofowora, 1993)	+	+	+

Key = + present; - = absent.

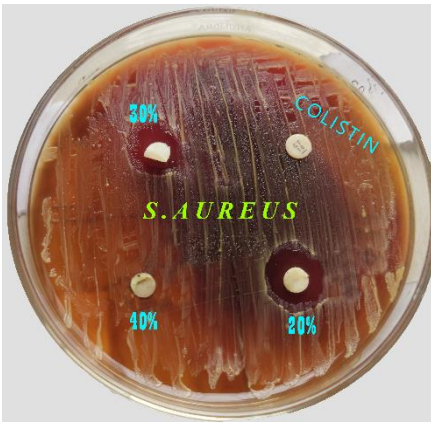

Figure 1(a). *Staphylococcus aureus* with 14 days (S1) Tulsi extract

Figure 1(b). *Salmonella* spp. with 14 days (S1) Tulsi extract

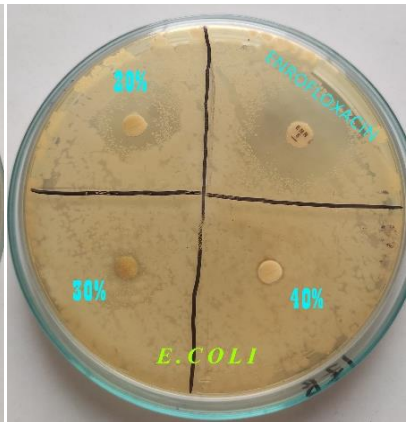

Figure 1(c). *E. coli* with 14 days (S1) Tulsi extract

Table 2. Presence of phytochemicals in three different extracts of *Swertia chirayita* (Chirota)

Phytochemicals	Test	7 Days extract	14 Days extract	21 Days extract
Alkaloids	Mayer's (Sofowora, 1993)	+	+	+
Flavonoids	Alkaline Reagent Test (Sofowora, 1993)	+	+	+
Saponins	Frothing (Sofowora, 1993)	-	+	+
Tannins	Lead acetate Test (Sofowora, 1993)	+	+	+
Phenolic compound	Ferric Chloride Test (Sofowora, 1993)	-	+	-
Glycosides	Killer killani Test (Sofowora, 1993) Borntranger's Test (Sofowora, 1993)	-	+	+
Carbohydrates	Molish's Test (Sofowora, 1993)	+	+	+
Reducing sugar	Benedict's Test (Sofowora, 1993) Feheling's Solution Test (Sofowora, 1993)	+	+	+
Protein and amino acid	Xantoproteic Test (Sofowora, 1993)	-	+	+
Acidic Compound	Sodium Bicarbonet Test (Sofowora, 1993)	-	-	-
Phytosterol	Libermann-Burchad's Test (Sofowora, 1993)	+	+	+
Steroids and terpenes	Salkowski Test (Sofowora, 1993)	+	+	+

Key = + present; - = absent.

Table 3. Zone of inhibition (mm) of *Ocimum sanctum* ethanolic extracts against *Escherichiacoli*, *Salmonella*spp. and *Staphylococcus aureus* at three different concentrations with 14- and 21-days extracts.

Bacterial isolates		*E. coli* (n=2)		*Salmonella* spp. (n=2)		*Staphylococcus aureus* (n=2)	
		Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD
0.2 mg/µL	S_1	7-9	7.7±0.75	14-16	15±0.8	14-15	14.3±0.44
	S_2	8-9	8.4±0.49	17-19	18.1±0.87	16-17	16.6±0.44
0.3 mg/µL	S_1	0	0	5-7	6.1±0.87	11-13	12.2±0.75
	S_2	0	0	12-14	13.1±0.69	13-16	14.6±1.19
0.4 mg/µL	S_1	0	0	4.5-5	4.7±0.26	0	0
	S_2	0	0	8-9	8.5±0.49	11-14	12.5±1.23

S_1= 14 days, S_2= 21 days

In Table 4 reveals that amoxicillin and sulfamethoxazole showed 100% sensitivity against *E. coli*, *Salmonella spp.* and *Staphylococcus aureus*. Colistin did not show sensitivity against all the isolates. Enrofloxacin showed 80% sensitivity to *E. coli* and *Staphylococcus aureus.* These anti-biogram properties were categorized according to the CLSI guideline 2019.

Table 4. The sensitivity and resistant pattern of different commercial antibiotics against *E. coli*, *Salmonella* spp. and *Staphylococcus aureus*

Antimicrobial	No	Ciprofloxacin	Amoxicillin	Enrofloxacin	Colistin	Sulfamethoxazole
Sample of *E. coli*	1	27 (S)	17 (S)	22 (I)	0	19 (S)
	2	28 (S)	18 (S)	24 (S)	0	22 (S)
	3	31 (S)	19 (S)	25 (S)	0	21.5 (S)
	4	31 (S)	19 (S)	26 (S)	0	21 (S)
	5	30.5 (S)	19.5 (S)	24.5 (S)	0	20.5 (S)
Sensitivity		100% S	100% S	80% S, 20% I	100% R	100% S
Sample of *Salmonella* spp.	1	23 (I)	20 (S)	27 (S)	0	22 (S)
	2	24.5 (I)	20.5 (S)	28 (S)	0	22.5 (S)
	3	25.5 (I)	20 (S)	29.5 (S)	0	23.5 (S)
	4	27 (S)	21 (S)	30 (S)	0	23 (S)
	5	26 (S)	21 (S)	28.5 (S)	0	25 (S)
Sensitivity		40% S, 60% I	100% S	100% S	100% R	100% S
Sample of *Staphylococcus aureus*	1	22 (S)	15	22 (I)	0	16
	2	22.5 (S)	15.5	23.5 (S)	0	16.5
	3	23 (S)	15.5	24.5 (S)	0	18.5
	4	24 (S)	16	26 (S)	0	17
	5	25 (S)	15	25.5 (S)	0	19
Sensitivity		100% S	100% S	80% S, 20% I	100% R	100% S

Note: S- Sensitive, I- Intermediary Sensitive, R- Resistant (CLSI, 2019)
Ciprofloxacin (S≥=26, I=22-25, R=≤21), Amoxicillin (S≥=15, I=12-14, R=≤11), Enrofloxacin (S≥=23, I=19-22, R=≤18), Sulfamethoxazole (S≥=16, I=11-15, R=≤10) `

4. DISCUSSION

As far as we are aware of, this probably be the first research attempt made to reveal phytochemical screening of plant extracts, including *Ocimum sanctum* (Tulsi) and *Swertia chirayita* (Chirota), and to assess their antimicrobial efficacy against *Salmonella* spp., *E. coli* and *Staphylococcus aureus* with different commercial antibiotics such as ciprofloxacin, amoxicillin, enrofloxacin, colistin, and sulfamethoxazole in Bangladesh.

According to the study, these leaf ethanolic extracts include a variety of secondary metabolites, including alkaloids, carbohydrate, tannin, flavonoids, saponins, phytosterols, glycosides, and terpenoids. Eugenol, methyleugenol, and caryophylllene are among the chemicals found in *Ocimum sanctum* leaves that are water-soluble phenolic compounds and may have immunostimulatory properties. According to Bairwa et al. (2012), saponins have anti-hyperlipidemic, hypotensive, and cardio-depressant effects. Alkaloids, tannins, flavanoids, steroids, phenols, and other aromatic compounds are only a few examples of the phytochemical components that plants produce to protect themselves against herbivores, insects, and other predators (Bonjar et al., 2004). In heart failure, glycosides can function as cardio stimulants (Sood et al., 2005). According to Asquith et al. (1986), tannins have antidiarrheal and hemostasis effects. The antioxidant and immune stimulatory effects are due to flavonoids. Significant analgesic, antipyretic, anti-inflammatory, and antibacterial properties were demonstrated by tulsi oil. According to Rajesh et al. (2017), it has also demonstrated memory-improving, antifertility, anticataract, antithyroid, antiulcer, antidiabetic, antiarthritic, anti-amnesia, antihelmenthic, hepatoprotective, and no tropical action. Alcoholic extract improved acetylcholinesterase inhibition and step-down latency and is therefore utilized to treat cognitive problems. Some plants, such as alkaloids, flavonoids, glycosides, and saponins, have antibiotic principles, which are the defensive mechanisms of the plants against pathogens, claim Craig et al. (1999) and Khanna et al. (2003).

According to Santra et al. (2017), *Ocimum sanctum* leaf extract had an impact on both

specific and general immune responses as well as disease resistance to bacterial and fungal infection. Both the antibody response and neutrophil activity were boosted. Experimental research has demonstrated the antibacterial effects of the ethanolic extract of *Ocimum sanctum*. Antimicrobial agents are produced by using medicinal herbs. The herb *Ocimum sanctum* has long been used in traditional medicine. Consequently, this plant's phyto-chemicals can treat various human illnesses. The herbs are inexpensive, widely accessible, and safe for customers, the environment, and living things, making them highly beneficial to living things (Musara et al., 2021).

The intensification of animal production because of the rising demand for goods derived from animals has increased the total usage of antibiotics in recent decades. Additionally, the quantity of antimicrobials utilization are being raised when diseases are being targeted, when a specific disease is being prevented from spreading, and when there is a high-stress level (Studdert et al., 2010). Antibiotics should be used carefully to treat animal infections since abuse and overuse can develop antibiotic resistance. The availability of antimicrobial medications for therapeutic use in terrestrial animals is crucial for the health, welfare, and production of the animals. It also helps to promote public health, food security, and food safety, which in turn helps to safeguard livelihoods. These advantages could be reversed by the increasing animal resistance to antimicrobial medications, which renders treatments ineffective, worsens illness severity, lowers productivity, and causes economic losses (Parle and Bansal, 2006).

Ocimum sanctum's potency and antibacterial properties were examined, considering the consequences for public health and the need to prevent antibiotic resistance in commercial chicken operations. *Ocimum sanctum*'s anti-microbial screening revealed that dosages of 0.2 mg/L for 14 days (S1) and 21 days (S2) had the largest zones of inhibition compared to other doses against *E. coli*, *Salmonella* spp., and *Staphylococcus aureus* in this study. The extracts of 14 days (S1) at a dosage of 0.2 mg/L produced the highest zone of inhibition against *Salmonella* spp. of any extract tested. The maximum zone of inhibition, as determined by Jacob et al. (2015), was 6 mm at 0.25 mg/L, which is less than the zone determined by the current study (19 mm at 0.2 mg/L).Another study, Narasimha et al. (2012) showed that the zone of inhibition of *S. aureus* found 22 mm, which is higher than the current study (17 mm). The disc containing 0.3 mg/µL and 0.4 mg/µL could not produce any zone of inhibition against *E. coli* isolates. This difference could result from varying *Ocimum sanctum* leaf quality or extraction efficiency.

Amoxicillin and sulfamethoxazole, two common medications, shown 100% sensitivity to *E. coli*, *Salmonella* spp., and *Staphylococcus aureus*. Enrofloxacin had shown 80% sensitivity to *Staphylococcus aureus* and *E. coli*. However, Colistin did not show sensitivity against all the isolates. Possible causes of the resistance include improper use of commercial antibiotics in chickens. Numerous causes, such as the improper use of antibiotics by medical experts, untrained practitioners, and the accessibility of antibiotics over the counter, among others, may have attributed to this resistance level. Antibiotics are frequently purchased in Bangladesh without a prescription, which encourages public usage and aids in the formation and spread of antimicrobial resistance in society (Islam, 2008). The current study has indicated that some bacterial isolates showed 100% resistance, similar to Cardoso et al., 2006 who found 100% resistance in *Salmonella* spp. and *E. coli*. Antimicrobial resistance patterns may differ depending on the species of bacteria isolated, the antimicrobials employed, the location of the farms, and other factors.

5. CONCLUSION

In *O. sanctum*, tannins and reducing sugar on day 7, tannins and acidic compound on day 14, and acidic compound on day 21 of extracts were absent. In *S. chirality*, saponins, phenolic compounds, glycosides, proteins, and amino acids, acidic compounds on day 7, acidic compounds on day 14, and phenolic and acidic compounds on day 21 of extracts were absent. Colistin sulfate showed 100% resistance to *E. coli*, *Salmonella* spp., and *Staphylococcus aureus*. And *E. coli* (20%) and *Salmonella* spp. (17%) isolates

from the broiler showed sensitivity to *O. sanctum* extracts. Finally, it might be concluded that 0.2 mg/ µL of *O. sanctum* extracts showed an intermediary zone of sensitivity against *E. coli*, *Salmonella* spp. and *Staphylococcus aureus.*

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