International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
Impact Factor: RJIF 5.45
IJBS 2024; 6(1): 18-23
www.biologyjournal.net
Received: 11-11-2023
Accepted: 15-12-2023

All author's names are given below the references

Antibacterial effects of lemon grass and tulsi on gram-negative bacteria

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DOI: https://dx.doi.org/10.33545/26649926.2024.v6.i1a.178

Abstract
In the developing world, medicinal plants are used to cure a wide range of illnesses. Research on them may result in the development of innovative medications for the efficient treatment of a range of illnesses. For this investigation, we selected the two most widely utilized plant leaves *Cymbopogon citrates* and *Ocimum tenuiflorum* after screening them for phytochemical and antibacterial qualities. The agar well diffusion method was used to qualitatively assess the antibacterial activity test against specific Gram-negative bacteria on a solid medium**.** According to the study, secondary metabolites including tannins, flavonoids, saponins, glycosides, alkaloids, and steroids were abundantly available in both *Cymbopogon citrate* extracts and *Ocimum tenuiflorum*. Additionally, it was discovered that plant leaf extracts exhibited the greatest inhibition against *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexineri*, and *Salmonella typhi*, as well as antibacterial qualities for a variety of gram-negative bacterial strains.

Keywords: Antimicrobial activity, *cymbopogon citratus*, *ocimum tenuiflorum*, medicinal plants, diffusion method

Introduction
For thousands of years, nature has provided medical remedies. In 80% of the world's impoverished and emerging nations, plant-based systems are still crucial to primary healthcare (Kalpana *et al*., 2011) [29]. Several bioactive chemicals in lemon grass provide therapeutic properties. Its ethnopharmacological uses have a lot of evidence supporting them (Kumar R *et al*., 2010) [30]. Over two-thirds of people in poor nations believe that herbal medicine plays a significant role in the healthcare system, according to the World Health Organisation. Due to its wide range, *Cymbopogon citrates* (*C. citrates*) is also referred to as lemongrass, barbed wire grass, fever grass, citronella grass, and tanglad (Karpagam *et al*., 2016; Oladeji *et al*., 2019) [10, 18]. Worldwide, rheumatism, fevers, menstrual irregularities, digestive diseases, and other joint ailments are all treated with calcium citrate (Simon JE. *et al*., 1984) [21].
The leaves of *C. citrates* are rich in bioactive compounds. The phytochemicals that have been isolated and identified from these leaves primarily consist of flavonoids, alkaloids, saponins, tannins, and phenolic compounds. These include kaempferol, luteolin, apiginin, quercetin, and iso orientin 2'-O-rhamnoside. These compounds are well-known for their numerous health, culinary, and pharmaceutical applications (Negrelle and Gomes, 2007; Hasim *et al*., 2015; Erminawati *et al*., 2019) [6, 9, 17]. For thousands of years, Ayurveda has utilized *Ocimum tenuiflorum*, popularly known as Tulsi, for its many therapeutic benefits. Known as "The Queen of Herbs" and the "Incomparable One" of India, Tulsi is highly revered and considered one of the most sacred herbs in the Orient, known for its numerous health benefits. Notable for its religious and spiritual sanctity, the sacred basil, or Tulsi, plays a significant part in the Eastern herbal medicine and holistic health systems of Ayurveda and Unani (Warrier, 1995) [26].
Ayurvedic treatments with tulsi extracts include treating heart disease, common colds, headaches, stomach problems, inflammation, poisonings, and malaria (Biswas *et al*., 2005) [3]. Tulsi is utilized traditionally in a variety of ways. For example, aqueous extracts from the leaves, either fresh or dried and powdered, are added to herbal teas or combined with other

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herbs or honey to increase their therapeutic efficacy. Aqueous Tulsi extracts have long been used to treat a variety of ailments, such as heart disease, inflammation, stomach aches, migraines, malaria, and common colds (Pattanayak *et al*., 2010) [31]. According to Committee A. P. (2016), there have been other documented medicinal applications for treating epilepsy, dyspnea or asthma, hiccups, cough, skin and hematological disorders, neuralgia, headaches, wounds, or inflammation. Since the start of the COVID-19 pandemic, numerous studies have been carried out in this area. Turmeric's antiviral and immunity-boosting qualities led to the expectation that the plant's flavonoids and polyphenolic acids would be beneficial as SARS-CoV-2 virus main protease inhibitors (Gautam S. *et al*., 2020) [32]. According to research conducted by Mohapatra PK. *et al*. (2020) [16], silico screening of tulsi phytochemicals against the SARS-CoV-2 virus's protease revealed that flavonoids and polyphenolic compounds, particularly luteolin-7-Oglucuronide and chlorogenic acid, may covalently bind to the main protease's active residue Cys145 and thereby irreversibly inhibit the viral enzyme.

Materials and Methods

Sample collection

All Tulsi and Lemon grass leaves were collected from the herbal garden of Krishna College of Science and Technology Bijnor (U.P). The plant leaves were healthy and free from any deformities (Fig 1). The leaves were brought to the laboratory for further processing.

Extraction preparation

The leaves of the tulsi and lemon grass should be well cleaned, let to dry, and then chopped into little pieces to make it into powder form. 100 milliliters of ethanol and water were used to soak 50 grams of Tulsi and lemon grass leaves for a full day. The filtrate can be collected and stored in a glass jar that has been sterilized. Use a shaking machine set to 100 rpm and filter using Whatman filter paper. Store the leftovers in the refrigerator at 4 °C for future use in experiments. Since ethanol is safe for ingestion by humans, and has long been recognized as an effective solvent for polyphenol extraction.

Proximate analysis

The air-dry method (Association of Official Analytical Chemists; AOAC 952.08, 2016) was used to quantify the proximate composition of the Tulsi leaf and lemon grass samples. According to Puwastien P. *et al*. (2011) [20], proximate analysis is used to estimate the quantitative quantity of food and food ingredients, such as moisture, ash content, total carbohydrates, and dietary fiber.

Qualitative analysis

Using the standard protocol outlined by Trease and Evans as well as Singh and Garg (2023) [22], a preliminary phytochemical analysis was performed to determine the bioactive chemical elements in the extracts of lemongrass and tulsi leaves. Except for the saponin test, which required 3 ml, 1 ml of each solvent extract was used to analyze each test.

Antimicrobial activity by agar well diffusion method

Using the agar well diffusion method, the antibacterial activity of Tulsi and Lemon grass was investigated. The chosen strains of bacteria were added to 10 milliliters of nutrient broth and incubated for a whole day at 37 ± 1 °C. Separately, 100 µL of each test bacterial strain was added to each Mueller Hinton agar plate, and the entire surface was aseptically spread out. Using a sterile crock borer with a 6mm diameter, the wells were cut aseptically, and 50µL of test extract from each leaf extract was added. The common Gram-negative pathogens *Escherichia coli*, *Shigella flexineri*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were used to measure the diameter of the zone of inhibition (in mm) on the upright plates after they were incubated for 24 to 48 hours at 37 ± 1 °C.

Results and Discussion

After the proximate composition analysis of lemongrass leaves was completed, the findings were tallied and presented in Table 1. The lemongrass plant's fresh green leaves were gathered, and proximate analysis was established. Lemongrass with a moisture level of 78% is ideal since it shields the plant from microbes and promotes a large amount of storage. It was discovered that leaves have 5.4% and 19.8% protein and carbohydrate content, respectively. This demonstrates the value of *Cymbopogon citrates* as an energy source. In that order, Lemongrass leaves have 3.2% fat, 9.5 percent fiber, and 7.4% ash content. Given its abundance in protein, antioxidants, and mineral nutrients like N (0.74%), P (0.07%), K (2.12%), S (0.19%), Mg (0.15%), Ca (0.36%), Zn (35.51 ppm), Mn (155.82%), and Fe (126.73%), as well as its richness in vitamins A, C, E, folate, niacin, and riboflavin, the lemongrass plant has a great deal of potential as food and fodder (Gaba J. *et al*., 2020 and Khandro A. *et al*., 2011) [7, 11].

In contrast, *Ocimum tenuiflorum* study indicates that the moisture content is 68%. Table 1 shows that the protein and carbohydrate content of Tulsi leaf is 8.89% and 58%, respectively, and the ash, fiber, and fat contents are 3.4%, 2.5%, and 2.4%, respectively. Tulsi can have trace levels of fats or lipids, which are an energy source and part of the plant's makeup (Kulkarni K and Adavirao BV., 2018) [12].

The data available (Table 2) indicates that alkaloids can be found in both the water and ethanol extracts of lemongrass. In contrast, they are only found in the water extract of Tulsi leaves, and when alkaloids are treated with ethanol, they become inactive. Alkaloids are mostly produced by biosynthesis from amino acids, which produce a range of chemical structures. These compounds are primarily extracted from plants (Verpoorte R. 2005) [25]. Alkaloids are particularly well-known as anesthetics, cardioprotective agents, and anti-inflammatory drugs in medicine. Prominent alkaloids that are utilized in medical environments comprise morphine, strychnine, quinine, ephedrine, and nicotine (Kurek J. 2015) [33].

The ethanol treatment of lemon grass results in an inactive form, however both the water and ethanol extracts of lemon glass and tulsi leaf contain saponin, tannins, and flavonoids (Table 2). Only a small percentage of the tens of thousands of phytochemicals present in plants have been extracted and identified (Cao *et al*., 2017; Singh and Chaudhuri, 2018) [4, 23]. According to Xiao (2017) and Zhao *et al*. (2018a) [27, 28], the most prevalent phytochemicals found in food are polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfurures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins,

phenylpropanoids, anthraquinones, ginsenosides, and so forth.

In this study, we analyze the antibacterial activity of several extracts of lemongrass and tulsi leaf against certain gram-negative pathogens, namely *E. Coli*, *Shigella*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, using the agar well diffusion method. (Tables 3, 4). According to this study, tulsi and lemongrass extracts have antibacterial properties that make them a potential mild antibiotic replacement. The nutritional value and antimicrobial activity of untreated and ethanol-treated lemongrass and tulsi herbs are determined by the presence of medicinally active constituents such as flavonoids, alkaloids, saponin, tannins, terpenoids, and glycosides (Mahendranathan, C and Abhayarathne, A., 2021; Benedek *et al*., 2022) [2, 15]. The results of this study demonstrate the antibacterial properties and health benefits of lemongrass and tulsi extracts. A plant that is frequently utilized is tulsi. The therapeutic qualities of tulsi and lemon grass are extensively utilized in the Ayurvedic pharmaceutical sector. The water extract of tulsi leaves has a 15 mm zone of inhibition against *E. coli*. Still, when the two are combined and dissolved in water for 24 hours, the extract displays an 18 mm zone of inhibition against *E. coli*, according to the data, which shows the high antimicrobial lemon grass (*Cymbopogon citratus*), which is used in Southeast Asian, Thai, and Vietnamese cuisines, among other regional variations. Several health issues, including constipation, coughs, diarrhea, elephantiasis, flu, headaches, gingivitis, malaria, leprosy, pneumonia, ophthalmia, stomach aches, and vascular disorders have been successfully treated with this highly regarded Brazilian traditional medicine (V. S. Nambiar and H. Matela, 2012) [24]. Table 4 presents results indicating that tulsi ethanol extract exhibits the largest zone of inhibition (18 mm) against *E. coli*. However, tulsi and lemon grass combined exhibit a 20.68 mm zone of inhibition against *Salmonella typhi* after being treated with ethanol. The tulsi plant has been shown in numerous experimental investigations to offer protection against the detrimental effects of various toxicants. According to some research, the tulsi plant guards against immune system reactions, industrial toxins, and pesticide-induced cell damage, which can impair the brain, kidneys, and liver. Accordingly, it was discovered that including tulsi in one's regular diet may help lessen the negative and toxic effects of common pesticides and industrial chemicals (M. M. Cohen, 2014) [14].

Table 1: Proximate analysis of *Cymbopogon citrates* and *Ocimum tenuiflorum*

Content	*Cymbopogon citrates*	*Ocimum tenuiflorum*
Moisture %	78%	68%
Ash %	7.4%	3.4%
Protein %	5.4%	8.89%
Fiber %	9.5%	2.5%
Carbohydrate %	19.8%	58%
Fat %	3.2%	2.4%

Table 2: Qualitative analysis of *Cymbopogon citrates* and *Ocimum tenuiflorum*

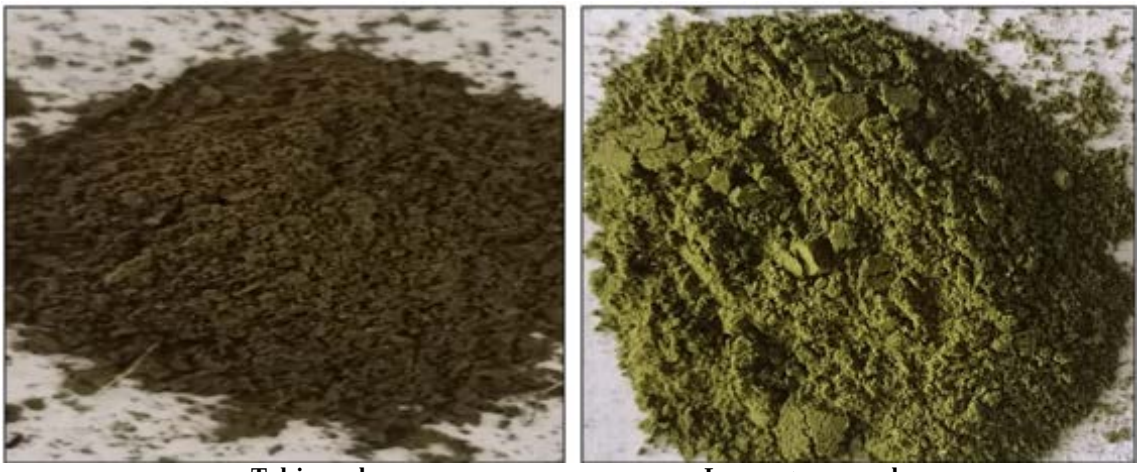
Phytochemical Nutrients	*Cymbopogon citrates* (Lemon grass leaf)		*Ocimum tenuiflorum* (Tulsi leaf)	
	Water extract	Ethanol extract	Water extract	Ethanol extract
Alkaloids	+	+	+	-
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	-	+	+
Steroids	-	+	+	+
Terpenoids	+	+	-	-
Glycosides	-	-	+	+

Table 3: Zone of inhibition of water extract of selective herbs against selective bacterial strains

Bacterial strains	Lemon grass	Tulsi	Lemon grass+ Tulsi
Escherichia coli	12 mm	15 mm	18 mm
Pseudomonas aeruginosa	14.3 mm	14 mm	15 mm
Shigella	11 mm	14 mm	15 mm
Salmonella typhi	10.5 mm	10.86 mm	14 mm

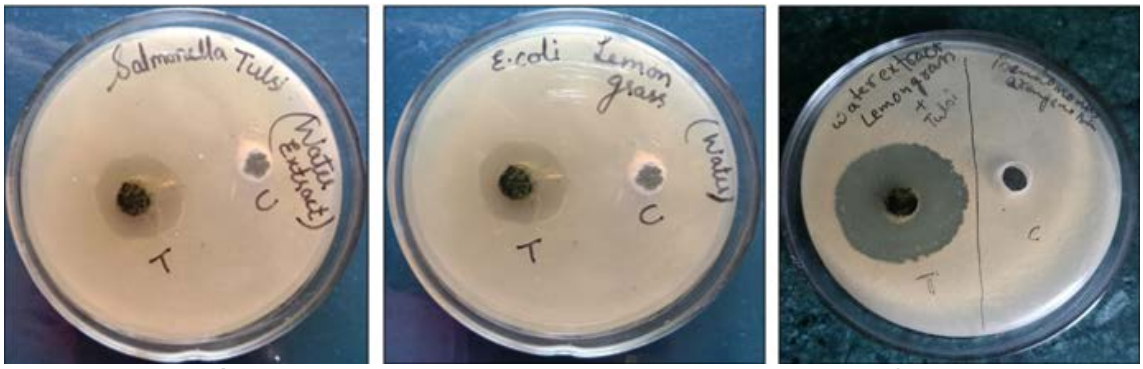
Table 4: Zone of inhibition of ethanol extract of selective herbs against selective bacterial strains

Bacterial strain	Control	Lemon grass	Tulsi	Lemon grass+ Tulsi
Escherichia coli	8.56 mm	14 mm	18 mm	20 mm
Pseudomonas aeruginosa	8.9 mm	16.5 mm	17.68 mm	18.6 mm
Shigella	7.4 mm	14.65 mm	17 mm	18 mm
Salmonella typhi	8 mm	13.68 mm	16 mm	20.68 mm



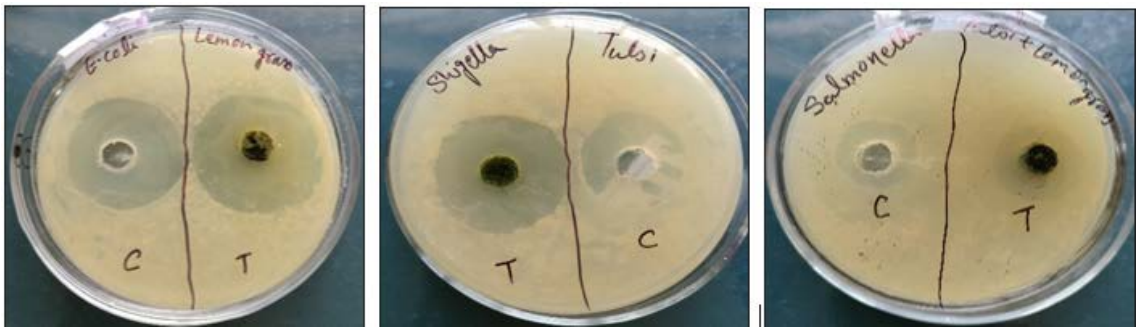
Tulsi powder **Lemon grass powder**

Fig 1: Powder form of Tulsi and Lemongrass



A **B** **C**

Fig 2: Different plates show zone of inhibition of water extracts against different test organisms where C denotes control of water and T denotes the test sample



A **B** **C**

Fig 3: Different plates show zone of inhibition of ethanol extracts against different test organisms where C denotes control of ethanol and T denotes the test sample

Conclusion

These components, which are not even provided by fruits and vegetables in the diet, are provided by culinary herbs. Even though they are a great source of many phytochemicals and certain important nutrients, people can only eat limited amounts of herbs. Its dietary contribution has comparatively little therapeutic impact because of this. If ingested daily, herbs may offer beneficial bioactive substances such as ubiquitous and less common phytochemicals. Herbs have been an integral element of Indian cooking and healing for thousands of years. Generally speaking, a lot of studies concentrated on its phytonutrients and antibacterial activities. The bioactive chemicals in herbs are more abundant when they are used as traditional medicines and ingredients than when they are eaten as plant flavorings in food. To maximize the health advantages of herbs, the ideal strategy is to use them "a little and often." It assisted in preventing numerous health issues rather than treating them.

Acknowledgments

The authors are thankful to Krishna College of Science and Information Technology Bijnor (U.P) India for providing facilities for conducting this work.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. AOAC International. Official Methods of Analysis of AOAC International. 20th ed. Gaithersburg, MD, USA: AOAC International; c2016. p.3172.
2. Benedek CS, Szakolczi1 O, Makai G, *et al*. Evaluation of physicochemical, sensory, and antimicrobial properties of small-scale produced fruit vinegar. Acta Alimentaria. 2022;51(1):1-10 DOI: 10.1556/066.2021.00077.
3. Biswas NP, Biswas AK. Evaluation of some leaf dusts as grain protectant against rice weevil *Sitophilus Oryzae* (Linn.). Environ Ecol. 2005;23:485-88.
4. Cao H, Chai TT, Wang X, Morais-Braga MFB, Yang JH, Wong FC, *et al*. Phytochemicals from fern species: Potential for medicine applications. *Phytochemistry Reviews*. 2017;16(3):379-440. DOI:10.1007/s11101-016-9488-7.
5. Committee AP. The Ayurvedic Pharmacopoeia of India, Part I, Volume IV. 1st. New Delhi, India: Government of India, Ministry of Health and Family Welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH); c2016.
6. Erminawati, Naufalin R, Sitoresmi I, Sidik W, Bachtiar A. Antioxidant activity of microencapsulated lemongrass (*Cymbopogon citratus*) extract. In: International Conference on Sustainable Agriculture for Rural Development (ICSARD 2018), Indonesia; c2018 Oct. p. 23-24, IOP Conference Series: Earth and Environmental Science (Volume 250). Purwokerto, IOP Publishing Ltd 2019, 012054-1-012054-6.
7. Gaba J, Bhardwaj G, Sharma A. Lemongrass. In: Nayik G.S., Gull A., editors. Antioxidants in Vegetables and Nuts-Properties and Health Benefits. Springer; Singapore: c2020. p. 75-103.
8. Gautam S, Gautam A, Chhetri S, Bhattarai U. Immunity against COVID-19: Potential role of Ayush Kwath. Journal of Ayurveda and Integrative Medicine. 2022;13(1):100350. DOI:10.1016/j.jaim.2020.08.003, PMID 32837101.
9. Hasim SF, Ayunda RD, Faridah DN. Potential of lemongrass leaves extract (*Cymbopogon citratus*) as prevention for oil oxidation. Journal of Chemical and Pharmaceutical Research. 2015;7(10):55-60.
10. Karpagam GN, Gayathri R, Vishnupriya V. Bioactivity analysis of lemongrass oil. Research Journal of Pharmacy and Technology. 2016;9(7):903-906.
11. Khandro A, Dhari AM, Panwar A, Naqvi B, Hussain G, Quaisar S, *et al*. Determination of different trace and essential element in lemon grass samples by x-ray fluorescence spectroscopy technique. Int. Food Res. J. 2011;18:265-270.
12. Kulkarni K, Adavirao BV. Indian traditional shrub Tulsi (*Ocimum sanctum*): The unique medicinal plant. Journal of Medicinal Plants Studies. 2018;6(2):106-110.
13. Kurek J. Alkaloids-Their Importance in Nature and Human Life. Intech Open; London, UK. Introductory Chapter: Alkaloids. Their Importance in Nature and for Human Life; c2019.
14. Cohen MM. Tulsi - *Ocimum sanctum*: A herb for all reasons, J Ayurveda. Integr, Med. 2014;5(4):251-59.
15. Mahendranathan C, Abhayarathne A. Antibacterial activities of some medicinal plants and green leafy vegetable: A Review. International Journal of Engineering Applied Sciences and Technology. 2021;6(4):78-84. http://www.ijeast.com.
16. Mohapatra PK, Chopdar KS, Dash GC, Raval MK. In silico screening of phytochemicals of *Ocimum sanctum* against main protease of SARS-CoV-2, ChemRxiv; c2020. DOI:10.26434/chemrxiv. 12599915. v1.
17. Negrelle RRB, Gomes EC. *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. Revista Brasileira de Plantas Medicinais Botucatu (Brasil). 2007;9(1):80-92.
18. Oladeji OS, Adelowo FE, Ayodele DT, Odelade KA. Phytochemistry and pharmacological activities of *Cymbopogon citratus*: A review. Scientific African. 2019;6: e00137.
19. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antibacterial activity of some plant essential oils. BMC Complement. Altern. Med. 2006;6:39. DOI: 10.1186/1472-6882-6-39
20. Puwastien P, Siong TE, Kantasubrata J, Craven G, Feliciano RR, Judprasong K. ASEAN Manual of Food Analysis. Institute of Nutrition, Mahidol University; c2011.
21. Simon JE, Chadwick AF, Cracker LE. The scientific literature on selected herbs and plants of the temperate zone, 2nd edition, Archon books, New York. Detection, evolution, and treatment of high blood cholesterol in adults. Int. Med. 1984;148:36-39.
22. Singh J, Amar P, Garg. Antimicrobial Activity of Sugarcane Vinegar with Eatables against Selected Food Borne Pathogens. Acta Scientific Microbiology. 2023;6(2);02-08.
23. Singh D, Chaudhuri PK. A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.). Industrial Crops and Products. 2018;118:367-82. DOI: 10.1016/j.indcrop.2018.03.048.
24. Nambiar VS, Matela H. Potential Functions of Lemon Grass (*Cymbopogon citratus*) in Health and Disease. Inter. J. Pharma. & Bio. Archives. 2012;3(5):1035-43.
25. Verpoorte R. Encyclopedia of Analytical Science. Elsevier; Amsterdam, The Netherlands: ALKALOIDS; c2005. p. 56-61.
26. Warrier PK. In: Longman O, editor. Indian Medicinal Plants. New Delhi, CBS publication; c1995. p.168.
27. Xiao JB. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Critical Reviews in Food Science and Nutrition. 2017;57:1874-905.
28. Zhao CCF, Yang B, Liu L, Lin SD, Sarker L, Nahar H, *et al*. Bioactive compounds from marine macroalgae and their hypoglycemic benefits. Trends in Food Science & Technology. 2018;72:1-12. DOI:10.1016/j.tifs.2017.12.001.
29. Kalpana KB, Devipriya N, Srinivasan M, Vishwanathan P, Thayalan K, Menon VP. Evaluating the radioprotective effect of hesperidin in the liver of Swiss albino mice. European journal of pharmacology. 2011 May 11;658(2-3):206-12.
30. Kumar S, Kumar R, Mehrotra SP. Influence of granulated blast furnace slag on the reaction, structure and properties of fly ash based geopolymer. Journal of materials science. 2010 Feb;45:607-15.
31. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic

applications: An overview. Pharmacognosy reviews. 2010 Jan;4(7):95.
32. Gautam S. COVID-19: air pollution remains low as people stay at home. Air Quality, Atmosphere & Health. 2020 Jul;13:853-7.
33. Labaj AL, Kurek J, Jeziorski A, Smol JP. Elevated metal concentrations inhibit biological recovery of C ladocera in previously acidified boreal lakes. Freshwater Biology. 2015 Feb;60(2):347-59.

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