WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 5, 1048-1061. Research Article ISSN 2277– 7105

SECONDARY METABOLITES SCREENING AND PHARMACOLOGICAL ACTIVITIES OF LEMONGRASS

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Article Received on
24 Jan. 2022,

Revised on 13 Feb. 2023,
Accepted on 05 March 2023

DOI: 10.20959/wjpr20235-27420

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ABSTRACT

The prominent cultivation of lemongrass (*Cymbopogon spp*.) relies on the pharmacological incentives of its essential leaf extraction. Dried leaves extract was prepared in aqueous and methanol solvent. Both Qualitative and Quantitative tests are done for confirmation of presence of phytochemicals in lemongrass. After, experiment it is observed that lemongrass leaves carry a remarkable and more amount of numerous bioactive compounds in methanol extract than in aqueous extract, such as Flavonoid (0.12g/100g), Tannin (0.063g/100g), Phenolics (0.02g/100g), Carbohydrate (0.16g/100g), Protein (0.02g/100g) and Alkaloid (1%). These crude plant extract contain various pharmacological properties including anti-inflammatory (69.39%) in aqueous extract & antioxidant (54%) in methanol extract. The antibacterial activity of the extract was estimated by using disc diffusion method, where inhibition zones are formed. In this paper it's observed the antibacterial property in *Staphylococcus aureus*, having inhibition zone of (22mm & 30mm) in methanol and aqueous extract respectively are observed, and *Escherichia coli*, having inhibition zone of (14mm) in methanol extract & no zone of inhibition in aqueous extract. So, in this paper it is analysed studied about the phytochemical screening and pharmacological activities of lemongrass. Lemongrass extract attributes are commercially exploited in the pharmaceutical, cosmetics, and food preservations industries.

KEYWORDS: Lemongrass, aromatic, medicinal, antioxidant, anti-inflammatory, antibacterial, phytochemicals.

INTRODUCTION

Lemongrass (*Cymbopogon citratus*) an aromatic herb, known in the North and West tropical Africa, in Arabian Peninsula and in Egypt, it was a native of (Southwest Asia) South India

but present, in many parts of the world growing in dense clumps. In the folk medicine of Brazil and Mexico (Uraku, 2015). *Cymbopogon citratus* staff were popularly known as citronella grass or lemongrass, this species belongs to the Gramineae family, which comprises approximately 500 genus and 8,000 herb species, the leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm (Manvitha & Bidya, 2014). Lemongrass (*Cymbopogon citrates*) plant leaves contained sufficient amounts of phytochemicals (alkaloids, glucosides, phenols, saponins, flavonoids, tannins, terpenoids and resins), steroids are absent. Chemicals contents of Lemongrass leaves are (Moisture, ash, fat, fiber, protein and Carbohydrate) (Khalifah et al., 2021).

Phytochemicals are biologically active naturally occurring secondary metabolites found in vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots. They are responsible for defending the plants against disease environmental stress, UV exposure etc. along with imparting colour, fragrance and flavour to the plant. The medicinal value of plant lies in the bioactive phytochemical constituents of the plant (Sheikh et al., 2013). The leaves and the oil are used to make medicine. Lemongrass is used for treating digestive tract spasms, stomach-ache, high blood pressure, convulsions, pain, vomiting, cough, achy joints (rheumatism), fever, the common cold, and exhaustion. It is also used to kill germs and as a mild astringent (Adegbegi et al., 2012; Naik et al., 2010).

In this paper we would discuss about the secondary metabolites content of the Lemongrass. The primary content of lemongrass detected are Flavonoid, Tannin, Phenols and alkaloid, which show bactericidal property, antioxidant property leads to anti-aging, anti-inflammatory, anticancer property having health benefits. Thus, lemongrass have medicinal values which by in future research would leads to important impacts on medicinal World. However, the application of Lemongrass in the treatment of cancer opens a new way in the field of therapeutics.

METHODOLOGY

Collection of plant material

The dried leaves of lemongrass (*Cymbopogon citratus*) were concurrently collected from Noida sector 12, Ashok plant nursery.

Preparation of Plant Extract

The dried lemongrass leaf sample are grinded in mortar and pestle to make fine powder. The powdered sample is then weighed. In 2gm of powdered sample, 10ml of methanol was added and kept for 2 days. Then it is filtered through Whatman's filter paper, thus labelled as sample1 (**M)** (methanolic sample). Again, in 2gm of powdered sample, 10ml of distilled water was added and filtered by same process by keeping 2 days and labelled as sample2 (**A**) (aqueous sample). The liquid extracts thus obtained were stored in refrigerator at 4 ^{o}c, thus used for further phytochemical analysis.

Fig 1: Samples (Lemongrass)

Fig 2 : Lemongrass Plant

Qualitative Analysis of Phytochemicals

The dried leaf extracts of lemongrass were analysed for the presence of phytoconstituents such as tannin, saponin, flavonoid, protein, alkaloid etc. according to the standard method. Phytochemicals which are tested for screening are Saponin (Froth test); Tannin (Braymer's test); Flavonoids : Alkaline reagent test, Lead acetate test, Ferric Chloride Test; Alkaloids test (Wagner's test); Cardiac Glycosides (Kellar-kiliani test); Terpenoids (Salkowski's test); Quinones (Sulphuric Acid test); Phenols : Iodine test, Lead acetate test, Potassium dichromate test; Coumarins (NaOH test); Resins (Turbidity test); Steroids (Acetic anhydride test); Phlobatannins (HCl test); Protein (Ninhydrin Test), (Biuret test); Carbohydrate (Barfoed's test).(Singh & Kumar, 2017; Uma et al., 2017; Audu et al., 2007; Gul et al., 2017;

Silva et al., 2017; Tiwari et al., 2011; Raaman, 2006; Maria et al., 2018; Kumar et al., 2018; Auwal et al., 2014).

Quantitative Analysis of Phytochemicals-

Quantitative Analysis of Phytochemicals was performed to determine the protein, carbohydrate, tannin, flavonoids and phenolics content in the respective plant extracts.

- **Tannin** content is estimated **by** (Van-Burden & Robinson, 1981). Tannin like compounds reduce phosphotungstic acid in alkaline solution to produce an intensely blue coloured solution, the intensity of which is proportional to the amount of tannin. Reagents used are 2ml of 0.1M $FeCl_3$, 0.008M $[K_4Fe(CN)_6]$ (Potassium ferrocyanide), 0.1N HCl and standard used is Tannic Acid (1mg/ml) in amount of 0.1ml to 0.4ml in 4 standard test tubes. O.D taken at 605 nm for blank, standard and sample test tubes.
- The principle behind **Protein** test is the reactivity of the copper (II) ions under alkaline conditions and the subsequent reduction of the Folin Ciocalteau phosphomolybdic phosphotungstic acid to heteromolybdenum blue, by the copper catalysed oxidation of aromatic acids. Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 to 10.5. Lowry method is sensitive to low concentration of protein and can detect concentrations as low as 0.05 mg/ml. Reagents used are FC reagent, $CuSO_4$(Copper sulfate), $KNaC_4H_4O_6{\cdot}4H_2O$ (Sodium Potassium tartrate), $NaCO_3$ (Sodium carbonate) and standard used is BSA(1mg/ml). O.D taken at 660 nm for blank, standard and sample test tubes. (Van Noorden et al., 2014; Kresge et al., 2005).
- Reducing sugar **Carbohydrate** is estimated by DNS method. Reducing sugars have the property to reduce many of reagents, one such reagent 3,5- dinitro salicylic acid which in alkaline solution is reduced to 3 amino 5 nitro salicylic acid. Reagents used are $KNaC_4H_4O_6{\cdot}4H_2O$ (Sodium Potassium tartrate), DNS, 2M NaOH and 1mg/ml glucose solution is used as standard solution. O.D taken at 540 nm for blank, standard and sample test tubes. (Hostettler, 1951).
- **Phenolics** possess a wide spectrum of biochemical activities such as antioxidants, antimutagenic, anticarcinogens as well as ability to modify the gene expression. Phenolics are the largest group of phytochemicals that account for the most of the antioxidant activity in plant products. The Folin-Ciocalteau Reagent (FCR) also called the gallic acid equivalent method (GAE) is a mixture of phosphomolybdate and phosphotungstate used

for the colorimetric in vitro assay of phenolic and polyphenolic antioxidant. Standard used is Gallic Acid solution(1mg/ml) and reagents are FC reagent, $NaCO_3$ (Sodium carbonate). O.D taken at 760 nm for blank, standard and sample test tubes. (Mallick & Singh, 1980).

- **Flavonoid** content is estimated by Aluminium Chloride method using Quercetin as standard solution (1mg/ml) was modified from the procedure reported. Quercetin which is the source of natural flavonoid content is used to make the calibration curve. Reagents used are 5% $NaNO_3$(0.3 ml), 10% AlCl3(0.3 ml) and 1N NaOH. O.D taken at 510nm for blank, standard and sample test tubes. (Kariyone et al., 1953).
- **Alkaloid** Lemongrass possesses alkaloid that contribute to high anti-inflammatory property. Alkaloids are a type of organic chemical that contains at least one nitrogen atom and is found in nature. Reagents used are 10% acetic acid (30ml), Ethanol and Liquid Ammonia. (Thorat et al., 2017). The whole solution settle down and precipited by Liq. Ammmonia and filterate is obtained. The filterate is then dried and weight to calculate the percentage of alkaloid. (Harborne, 1973).
- **Antioxidant property** Lemongrass possesses antioxidants that delays protective measures against reactive species. It's extracts can also defence the endogenous antioxidant defence system in alveolar macrophages cells through augmenting the superoxide dismutase activity and glutathione formation. Reagents used is 3ml of 2,2-diphenyl-1-picrylhydrazyl. O.D taken at 517 nm. (Brand-Williams et al., 1995).
- **Anti-inflammatory property** Anti-inflammatory substances can be an effective tool in the therapeutic treatment of the diseases. BSA test is done for anti-inflammatory detection test. Reagents used are Bovine Serum Albumin (1mg/ml), 1N HCl and phosphate buffer solution. O.D taken at 600 nm. (Kumar et al., 2018). Amount of BSA taken 0.45ml in each test tubes, pH maintain to 6.3 and then 5ml Phosphate buffer is added.
- **Antibacterial property** Lemongrass induces the destruction of bacterial biofilms and hinders further bacterial growth and development (Moore-Neibel et al., 2012). It's components can destabilise the bonds between the lipid bilayer and neutralise the bacteria through membrane disintegration (Kotzekidou et al., 2008). Antibacterial property is determined by AST method. Presence of inhibition zones would detect the antibacterial property against both *E.coli* and *Staphylococcus aureus* in both methanol and aqueous extract.

RESULTS

Phytochemical analysis is of paramount importance in identifying new source of therapeutically and industrially valuable compounds having including medicinal plants which have been chemically investigated. (Ambasta et al., 1986).

Qualitative Analysis of Phytochemicals-

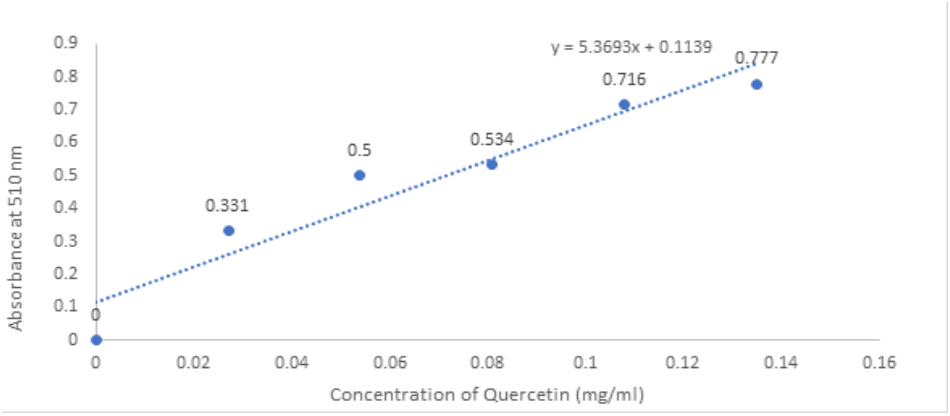
Table 1: Qualitative Analysis of Phytochemicals.

SL. No.	Phytochemicals	Methanol extract (M)	Aqueous extract (A)
1.	Saponin	-	Partially +
2.	Tannin	++	++
3.	Flavonoid	+	++
4.	Cardiac Glycosides	+	-
5.	Phenol	+	+/-
6.	Alkaloid	+	+
7.	Coumarins	+	+
8.	Resins	-	+/-
9.	Terpenoid	-	-
10.	Steroid	-	-
11.	Phlobatannins	-	-
12.	Protein	+	+
13.	Carbohydrate	-	-
14.	Quinones	-	-

++ = Strongly positive, + = Positive, - = Negative, +/- = Maybe present or absent

Quantitative Analysis of Phytochemicals

- **Flavonoid:** Flavonoid content in lemongrass highly contribute to antioxidant and anti-inflammatory properties. By, qualitative and quantitative test presence of Flavonoid is confirmed.

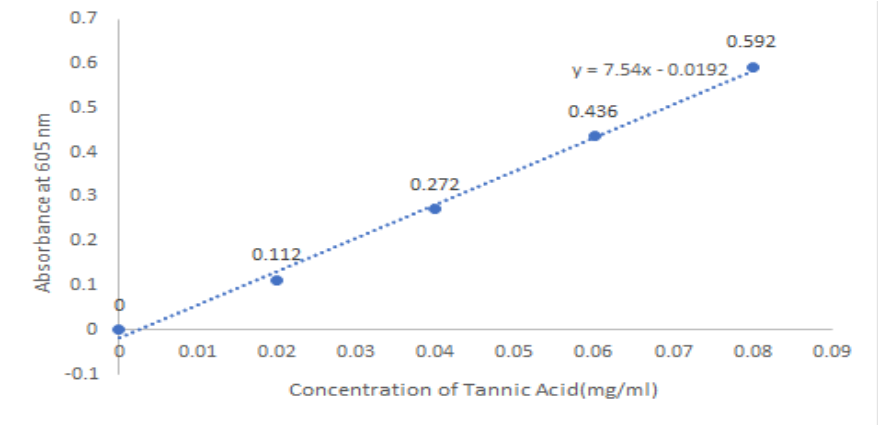


Graph 1: Standard graph of Quercetin.

$y = mx + c$

y = 5.3693x + 0.1139; (y of M_1 = 0.762, y of M_2 = 0.760, y of A_1 = 0.351, y of A_2 = 0.354)
x of M_1 = 0.241mg/ml, x of M_2 = 0.241mg/ml, x of A_1 = 0.088mg/ml, x of A_2 = 0.089 mg/ml.
(M = methanolic Extract, A = aqueous Extract).

- **Tannin:** Tannin content in lemongrass contribute to antioxidant and anti- inflammatory properties.

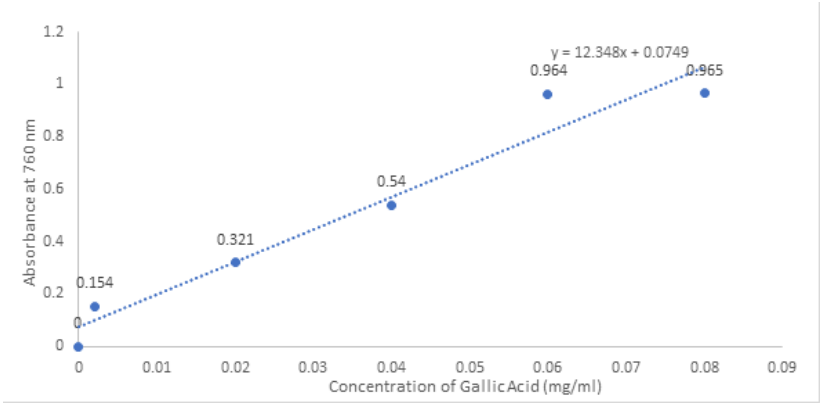


Graph 2: Standard graph of Tannic Acid.

y = mx + c

y = 7.54x + 0.0192; (y of M_1= 0.428, y of M_2 = 0.500, y of A_1 = 0.464, y of A_2 = 0.470)
x of M_1 = 0.108mg/ml, x of M_2 = 0.126mg/ml, x of A_1 = 0.116mg/ml, x of A_2 = 0.118mg/ml.
(M = methanolic Extract, A = aqueous Extract).

- **Phenolics:** Phenolics compounds are responsible for their chemo-preventive property.

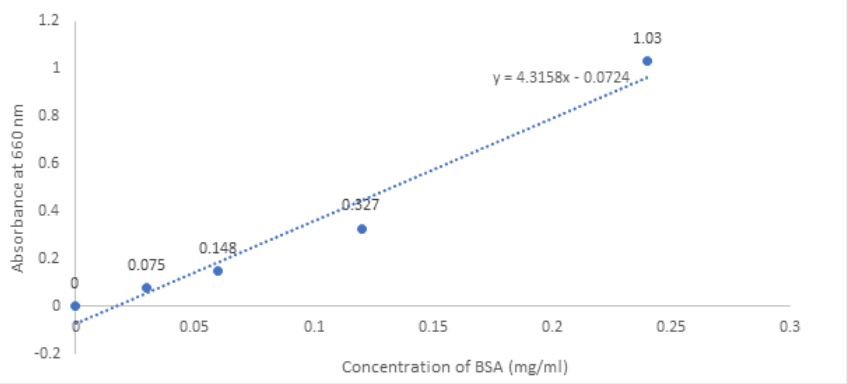


Graph 3: Standard Graph for Gallic Acid.

y = mx + c

y = 12.348x + 0.0749; (y of M_1 = 0.265, y of M_2 = 0.260, y of A_1 = 0.353, y of A_2 = 0.365)
x of M_1= 0.030mg/ml, x of M_2= 0.0.029mg/ml, x of A_1= 0.044mg/ml, x of A_2= 0.046mg/ml.
(M = methanolic Extract, A = aqueous Extract).

- **Protein:** Protein content contribute to anti-inflammatory property.



Graph 4: Standard Graph for BSA.

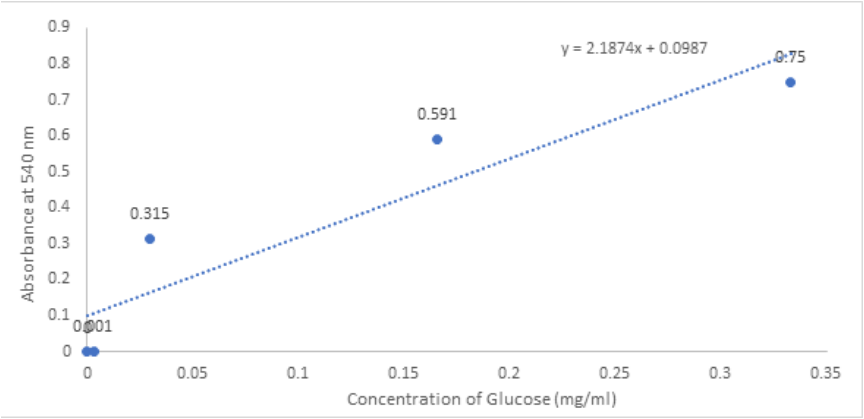
y = mx + c

y = 4.3158x + 0.0724; (y of M_1 = 0.155, y of M_2 = 0.165, y of A_1 = 0.126, y of A_2 = 0.130).

x of M_1= 0.038mg/ml, x of M_2= 0.042mg/ml, x of A_1= 0.024mg/ml, x of A_2= 0.026mg/ml.

(M = methanolic Extract, A = aqueous Extract).

- **Carbohydrate:** Carbohydrate content in lemongrass helps to reduce blood sugar level.



Graph 5: Standard Graph for Glucose.

y = mx + c

y = 2.1874x + 0.0987; (y of M_1= 0.450, y of M_2= 0.430, y of A_1= 0.423, y of A_2 = 0.435).

x of M_1= 0.32mg/ml, x of M_2 = 0.302mg/ml, x of A_1= 0.296mg/ml, x of A_2= 0.308mg/ml.

(M = methanolic Extract, A = aqueous Extract).

Quantitative contents of Phytochemicals

Table 2: Phytochemicals content of Lemongrass.

Sl. No.	Phytochemicals	Extracts	Values are the mean of two Independent analysis (g/100g) ± (S.D)
1.	Flavonoid	S(M)	0.120±0.0002
		S(A)	0.044±0.0004
2.	Tannin	S(M)	0.058±0.0063
		S(A)	0.058±0.0007
3.	Phenolics	S(M)	0.015±0.0003
		S(A)	0.022±0.0007
4.	Protein	S(M)	0.020±0.0017
		S(A)	0.012±0.0007
5.	Carbohydrate	S(M)	0.155±0.0064
		S(A)	0.151±0.0042

S(M): Methanolic Extract of Lemongrass, S(A): Aqueous extract of Lemongrass.

- **Alkaloid:** Percentage of alkaloid, present in methanol and aqueous extract are **0.5%** and **1%** respectively.

- **Antioxidant Property.**

Table 3: Antioxidant Property.

Test tubes	Optical Density at 517 nm
Blank	0.326
S_1(M)	0.148
S_2(A)	0.166

S(M): Methanolic Extract of Lemongrass, S(A): Aqueous extract of Lemongrass.

Percentage of antioxidant content in lemongrass is 54.60% and 49.07% in Methanol and Aqueous extract respectively.

- **Anti-inflammatory Property**

Table 4: Anti-inflammatory Property.

SL. No. Test Tubes	Optical Density at 600 nm
Blank	0.464
S_1(M)	0.273
S_2(A)	0.142

S(M): Methanolic Extract of Lemongrass, S(A): Aqueous extract of Lemongrass.

Percentage of anti-inflammatory content in lemongrass is 41.16% and 69.39% in methanol and aqueous extract respectively.

- **Antibacterial Property:** It is observed that antibacterial property of Lemongrass shown in *Escherichia coli*, shows zone of inhibition of 14mm in methanol extract however it's absence in aqueous extract. In *Staphylococcus aureus*, it shows inhibition zone of (22mm & 30mm) in methanol and aqueous extract respectively.

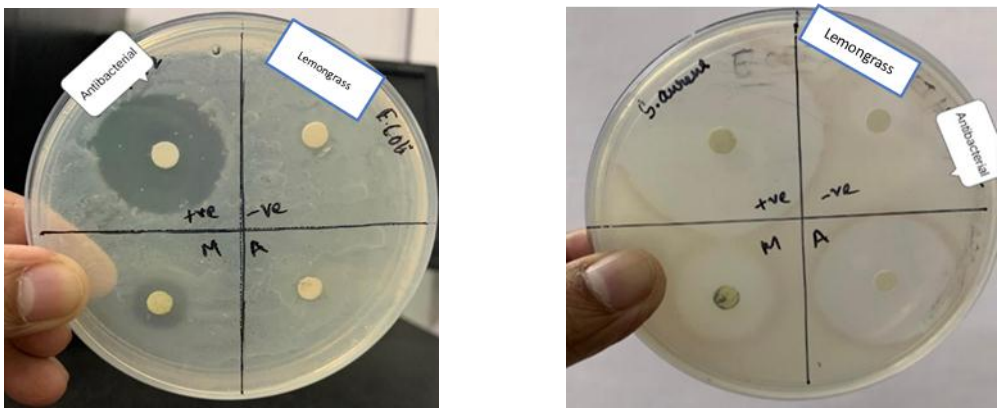


Fig 3: Test for Antibacterial Activity of Lemongrass on *Escherichia coli* and *Staphylococcus aureus.*

Table 5: Antibacterial Activity.

Quadrants	Inhibition B1(mm)	Inhibition B2(mm)
+ve	27	42
-ve	0	0
M	14	22
A	No any inhibition	30

+ve = Antibiotic control Ciprofloxacin, M = Methanol Extract, A = Aqueous Extract, -ve = Negative control. B1= *Escherichia coli* and B2= *Staphylococcus aureus*.

DISCUSSION

The aqueous and methanolic extract showed maximum number of plant constituents such as flavonoids, phenol, tannins, carbohydrate, glycosides, protein and amino acids as primary qualitative analysis of phytochemicals in leaves extract of lemongrass, thus it shows medicinal properties having health benefits. The quality of lemongrass essential oil is influenced by climatic conditions, seasons, age, lemongrass parts, and extraction conditions. (Hartatie et al., 2019). Using a solvent as an extraction medium will determine the polarity of the active compound in the extract. Different phytochemicals have been found to possess a wide range of medicinal properties, which may help in protection against various diseases, like alkaloids protect against chronic diseases. Contents of Flavonoid (0.12g/100g), Tannin (0.063g/100g), Phenolics (0.02g/100g), Carbohydrate (0.16g/100g), Protein (0.02g/100g) and

Alkaloid (1% are present) in methanol extract. The leaves of lemongrass extract have high carbohydrate content and it is concluded that *Cymbopogon citratus* is a very good source of energy (Özcan et al., 2009). The higher amount of phenol is important in regulation of plant growth, development and to make it disease resistance. The presence of tannin in lemon grass leaves is observed that contribute to various medicinal properties such as antimicrobial, anti-inflammatory and astringent activity (Gopinath & Suneetha, 2013). Adoption of BSA protein denaturation assay for invitro evaluation of anti-inflammatory potential of methanolic plant extracts circumvented associated with use of animals, in early stages for plants with potential lead anti-inflammatory compounds. In the present study it is clear that lemongrass possess a promising antibacterial activity against the test organisms. The results obtained from the Agar diffusion assay. Antibacterial property is observed in both gram positive and gram-negative test bacteria.

CONCLUSION

This study reveals the medicinal property of Lemongrass having therapeutic, aromatic and pharmacological activities. High content of flavonoids, tannin, phenolics contribute to antioxidant and anti-inflammatory property. Antioxidant helps to fights against free radicals that damages cells and acts against tumour cells, thus promotes anti-aging and anticancerous property. Presence of bioactive compounds, citral contribute anti-inflammatory property which leads to prevents skin inflammation, allergies, arthritis. Having bactericidal and bacteriostatic property, prevents prevent from blood infections, intestinal infection, skin infections. It also releases serotonin, which is effective effecting against headache and migraine, relieve minimise stress, anxiety by its aromatic property. So, it is concluded that lemongrass have contains high enourmous amount of medicinal property and it is suggested to includes more research on lemongrass, for future medicinal science, expecting fruitful utilization of it. Consideration also required in creating and mixing a product and also have an entrepreneurial thinking.

REFERENCE

1. Adegbegi, A.J., Usunobun, U.; Adewumi, BL.; Okungbowa, A.; Gabriel, OA. (2012). Comparative studies on the chemical composition and antimicrobial activities of the ethanolic extracts of lemon grass leaves and stems. *Asian J. Med. Sci, 4*(4): 145-148.
2. Ambasta, S. P. (1986). The useful plants of India, Publication and Information Directorate, CSIR. *New Delhi, India*.

3. Audu, S. A., Mohammed, I., & Kaita, H. A. (2007). Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Science Journal*, *4*(4): 75-79.
4. Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). In *Veterinary research forum: an international quarterly journal* (5): 2, 95). Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
5. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1): 25-30.
6. De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*, *5*(2): 29-32.
7. Gopinath, S. M., & Suneetha, T. B. (2013). Sumer Singh. Effect of *Cymbopogon citratus* terpenoids against bacterial pathogens causing bovine mastitis. In. *J Pharm and Bio sci*, *1:* 247-253.
8. Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal*, *2017*.
9. Harborne, J. B. (1973). Phytochemical methods chapman and Hall. *Ltd. London*, *4:* 49-188.
10. Hartatie E S., Prihartini I., Widodo W., and Wahyudi A (2019). Bioactive compounds of lemongrass (Cymbopogon citratus) essential oil from different parts of the plant and distillation methods as natural antioxidant in broiler meat *IOP Conf. Ser. Mater. Sci. Eng*, 532: 0–6.
11. Hostettler, F., Borel, E., & Deuel, H. (1951). Über die Reduktion der 3, 5- Dinitrosalicylsäure durch Zucker. *Helvetica Chimica Acta*, *34*(6): 2132-2139.
12. Kariyone, T., Hashimoto, Y., & Kimura, M. (1953). Microbial studies of plant components. IX. Distribution of flavonoids in plants by paper chromatography. *J Pharm Soc (Japan)*, *73:* 253-256.
13. Khalifah, A. M., Abdalla, S. A., Dosoky, W. M., Shehata, M. G., & Khalifah, M. M. (2021). Utilization of lemongrass essential oil supplementation on growth performance,

meat quality, blood traits and caecum microflora of growing quails. *Annals of Agricultural Sciences*, *66*(2): 169-175.

14. Kotzekidou, P., Giannakidis, P., & Boulamatsis, A. (2008). Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate. *LWT-Food Science and Technology*, *41*(1): 119-127.
15. Kresge, N., Simoni, R. D., & Hill, R. L. (2005). The most highly cited paper in publishing history: Protein determination by Oliver H. Lowry. *Journal of Biological Chemistry*, *280*(28): e26-e28.
16. Kumar, R., Sharma, S., & Devi, L. (2018). Investigation of total phenolic, flavonoid contents and antioxidant activity from extracts of *Azadirachta indica* of Bundelkhand Region. *Int. J. Life. Sci. Scienti. Res. eISSN*, *2455*(1716): 1716.
17. Kumar, V., Singh, S., Singh, A., Dixit, A. K., Shrivastava, B., Kondalkar, S.A., Singh, J., Singh, R., Sidhu, G.K., Singh, R.P. and Subhose, V., & Prakash, O. (2018). Determination of phytochemical, antioxidant, antimicrobial, and protein binding qualities of hydroethanolic extract of Celastrus paniculatus. *Journal of Applied Biology and Biotechnology*, *6*(6): 11-17.
18. Manvitha, K., & Bidya, B. (2014). Review on pharmacological activity of *Cymbopogon citratus*. *Int J Herb Med*, *6*: 7.
19. María, R., Shirley, M., Xavier, C., Jaime, S., David, V., Rosa, S., & Jodie, D. (2018). Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador. *Journal of King Saud University-Science*, *30*(4): 500-505.
20. Moore- Neibel, K., Gerber, C., Patel, J., Friedman, M., & Ravishankar, S. (2012). Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. *Journal of Applied Microbiology*, *112*(3): 485-492.
21. Naik, M. I., Fomda, B. A., Jaykumar, E., & Bhat, J. A. (2010). Antibacterial activity of lemongrass (*Cymbopogon citratus*) oil against some selected pathogenic bacteria. *Asian Pacific Journal of Tropical Medicine*, *3*(7): 535-538.
22. Özcan, M. M., Erel, Ö., & Herken, E. E. (2009). Antioxidant activity, phenolic content, and peroxide value of essential oil and extracts of some medicinal and aromatic plants used as condiments and herbal teas in Turkey. *Journal of medicinal food*, *12*(1): 198-202.
23. Raaman, N. (2006). Phytochemical techniques. *New India Publishing*, 19-24.

24. Sheikh, N., Kumar, Y., Misra, A. K., & Pfoze, L. (2013). Phytochemical screening to validate the ethnobotanical importance of root tubers of Dioscorea species of Meghalaya, North East India. *Journal of Medicinal Plants*, *1*(6): 62-69.
25. Singh, V., & Kumar, R. (2017). Study of phytochemical analysis and antioxidant activity of *Allium sativum* of Bundelkhand region. *International Journal of Life-Sciences Scientific Research*, *3*(6): 1451-1458.
26. Thorat, P., Sawate, A., Patil, B., & Kshirsagar, R. (2017). Effect of lemongrass powder on proximate and phytochemical content of herbal cookies. *Journal of Pharmacognosy and Phytochemistry*, *6*(6): 155-159.
27. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). A review: Phytochemical screening and extraction. *Internationale Pharmaceutica Sciencia*, 1(1): 98-106.
28. Uma, K. S., Parthiban, P., & Kalpana, S. (2017). Pharmacognostical and preliminary phytochemical screening of Aavaarai Vidhai Chooranam. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(10): 111-116.
29. Uraku, A. J. (2015). Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. *Research journal of phytochemistry*, *9*(4): 175-187.
30. Van Noorden, R., Maher, B., & Nuzzo, R. (2014). The top 100 papers. *Nature News*, *514*(7524), 550.
31. Van-Burden TP, Robinson WC (1981). Formation of complexes between protein and Tannin acid. J. *Agric. Food Chem*, 1: 77.