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## Research Article

# EXTRACTION, IDENTIFICATION, ESTIMATION OF PHYTOCONSTITUENTS AND PHARMACOLOGICAL EVALUATION OF AQUEOUS AND CHLOROFORM EXTRACT OF CYMPOBOGON CITRATUS (LEMON GRASS)

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### ABSTRACT

Phyto Chemistry is a fast growing study of Phyto Chemicals which are chemicals derived from plants.

The phyto chemical include the flavanoids terpenoids, lignans, polyphenolic etc. In the present study lemon grass was taken for many pharmacological activities. Lemon grass belongs to poacea family, lemon grass were collected, dried, powdered and extracted with water and chloroform. and Extraction done by soxhlet apparatus.

Separation of lemon grass extracts were done by column chromatography method. Phytochemical screening tests were carried out and analysis showed that it contain alkaloids, flavanoids, tannins and phenols. The constituted were estimated using electrometric method. Alkaloids estimated by "SINGHMBTH" method flavanoids estimated by ZHISHEN AlCl<sub>3</sub> Method. Phenols were estimated by FOLIN – CIOCALTEAU method and finally tannins by Prussian blue method. Concentration of the extracts were known and amount of phyto constituents were calculated.

Pharmacological evaluation of extracts done by Anti-Oxidant method Namely Nitric Oxide, hydroxyl free radical scavenging activity.

The Anti Bacterial activity were detected by disc diffusion method the dispenser method using species Ecoli and staphylococcus aureas. The result of analysis establish the pharmacological potential of lemon grass.

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## INTRODUCTION

### Lemongrass (*Cymbopogon Citratus*)

Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form. Plants have been identified as the potent therapeutic agent, due to the presence of nutritional (minerals and vitamins) and non-nutritional (fibres, active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides) component, hence promoted as "functional food".

Lemon grass *Cymbopogon citratus* is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Carlin, *et al.*, 1986). The plant is a native herb from India and is cultivated in other tropical and subtropical countries. (Figueirinha *et al* 2008).

Tissue inflammation is one of the main health issues worldwide. The hike in its prevalence has been attributed to sophisticated lifestyles occasioned by technological advancement. Therefore, inflammation accounts for more cases of mortality among people. Importantly, it has been linked with other health problems like cancer, cardiovascular rheumatoid, neurodegenerative and diabetes (Jaswir and Monsur, 2011).

Solvent extracts, polyphenol rich extractants and citral isolate are the chief components of lemongrass exhibiting anti-inflammatory activities as reported by several investigators. Similarly, aqueous extracts devoid of lipid and essential oil and polyphenol fractions (phenolic acids, flavonoids and tannins) of lemongrass leaves were investigated for their anti-inflammatory properties. The mechanism of action is by inhibiting the secretion of NO and pro-inflammatory cytokine tumor necrosis factor TNF- $\alpha$  (Tiwari *et al.*, 2010).

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Oxidation is a fundamental process in human cells, tissue and systems leading to formation of reactive oxygen species (ROS) which include hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide anion (O<sup>2-</sup>) and free radicals (Heoet al., 2003). Antioxidants have to be present in the body to offer protective mechanism against damaging effects of oxidation process caused by these radicals (Finkel, 1998; Thannickal and Fanburg, 2000).

Anti-bacterial activity in extracts of plant materials has been elucidated from various sources in recent times with promising results. These components demonstrate their antibacterial activity by inhibiting the growth of both Gram positive and Gram negative bacteria. Several investigations have been carried out on the potentials of lemon grass extract as a source of hypolipidemic and hypoglycemic substances which may lower the risks of hypertension and obesity. The presence of anti-hypertensive compounds such as flavonoids and alkaloids has been reported to assist in the hypoglycemic properties exhibited by lemon grass aqueous extract since it contains essential oil and other extractants (Onabanjoet al., 1993; Oladele et al., 1995). In order to elucidate its efficacy in curing anxiety related conditions, researchers have investigated anxiolytic properties of lemongrass tea (Liberalliet al., 1946; Alves et al., 1960; Olaniyiet al., 1975; Nogueira, 1983). The results showed that both leaves and stalk extracts possess radical scavenging ability in a dose dependent manner (Mirghaniet al., 2012). The possibility of lemon tea possessing antinociceptive effects has been well researched over the years. Earlier reports showed that lemon grass extracts has little or no positive actions thereby negating the claims in folk medicine (Carliniet al., 1986; Leiteet al., 1986; Souza-Formigoniet al., 1986). The action of essential oils extracted from lemon grass decoction against both pathogenic and edible fungi is of immense contribution as investigated by researchers. Lemon grass oil showed a promising prospect among several essential oils by inhibiting the growth of fungi cells which are implicated in secreting mycotoxins during storage of grains and other food products (Fandohanet al., 2008; Nguefackaet al., 2012). Here, the synergistic effects of oil fractions showed both synergistic and antagonistic effects among different portion of characterized oils (Viana et al., 2000; Nguefackaet al., 2012). Several studies (both *in-vivo* and *in-vitro*) have been conducted to investigate cytotoxicity and mutagenicity effects of lemongrass extract in order to confirm the safety of lemongrass tea. All phenolic compounds isolated from methanolic extract of lemongrass were nontoxic to human lung fibroblasts even at high concentration (1 mM) (Cheelet al., 2005). Although, slight increase in bilirubin and amylase in some of the volunteers observed, such increase did not exhibit any medical implication. Furthermore, lemongrass tea did not show any hypnotic and anxiolytic properties (Leiteet al., 1986). essential oil from lemongrass is safe for human consumption and can be used for maize storage at prescribe concentration (Fandohanet al., 2008).

### **The Scope of the Present Work**

Pyto chemistry is a fast growing area of research various including chemistry physics biology and medicine .Lemongrass had a phytoconstituents. The Estimation of pytoconstituents carried out it has been used as a pharmacological evolution studies

## **MATERIALS AND METHODS**

The plant was collected from Chitannavassel rock near Pudukottai district of, Tamilnadu and identified by the botanist of Sri Sairam Siddha Medical College, Tambaram, Chennai and a specimen was kept in their laboratory. The collected plant material was allowed to dry in sunshade for a week and after that it was crushed and powdered and soaked in chloroform for 3 days (72 hrs). The extract were done by soxhlet apparatus and prepared chloroform and aqueous extract then it is separated by columns chromatography method.

### **Test for Alkaloids**

#### **Mayers test**

2 ml of the extract was added with Meyers reagent (1.36 g Mercuric chloride + 3.0 gm KI in 100 ml of water). Appearance of greyish white precipitate indicates the presence of alkaloids. Morquies, Dragendorffs, Hayers, Wagnerstest were also carried out brown precipitate indicates presents of Alkaloids

#### **Test For Flavanoids (shinoda's test)**

2 ml of extractl was warmed and to the warmed solution a piece of Magnesium ribbon was added followed by 2 drops of concentrated HCl drop by drop. Absence of orange or yellow colour indicates the presence of flavanoids.

#### **Test For Phenols OH Group (FeCl<sub>3</sub> test)**

2 ml of extract was warmed then 2 drops of neutral ferric chloride and the colour was observed. Presence of brown green colour indicates the presence of phenolic hydroxyl group.

#### **Test for Tannins**

2 ml of extract in 20 ml of water in a test tube. Filter the above mixture add few drops of 0.1% ferric chloride. Development of a brownish green or a blue-black colouration indicated the presence of tannins.

The results of the above tests are showed in TABLE 1(phyto chemical screening test).

### **Estimation of Phyto Constituents**

#### **Alkaloid Estimated By Singh MBTH Method**

##### **Procedure**

1. 0.2,0.3,0.4,0.5,0.6 ml of standard solution was pipetted out in to a series of 50 ml standard flasks.
2. 2 ml of the sample was pipetted out in a separate 50 ml standard flask.
3. To all the flask including the blank 1 ml of standard were added and 0.5 ml of 0.1 M acid.
4. All the flasks were kept in a boiling water bath for 10 minutes.
5. 2 ml of 0.01M MBTH was added into all the flasks and boiled in a water bath for 2 minutes.
6. All the flasks were cooled and made up to the mark with distilled water.
7. The Optical Density (OD) of the blue colour formed was measured at 630 nm for all the sample.

- A graph was plotted by taking concentration of Theophylline along X-axis and optical density along Y-axis.
- From the standard curve on the graph, the concentration of unknown sample was calculated.

**In the same way following phytoconstituents were estimated**

Flavanoids Estimated By Zhishen AlCl<sub>3</sub> Method

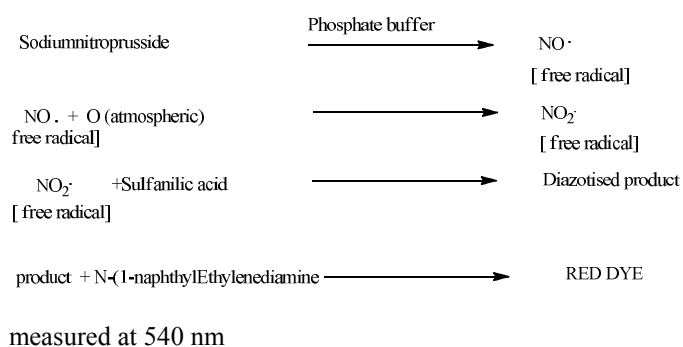
Total Phenolics By Folin –Ciocalteu Method

Tanins By Prussian Blue Method

**Pharmacological Evaluation**

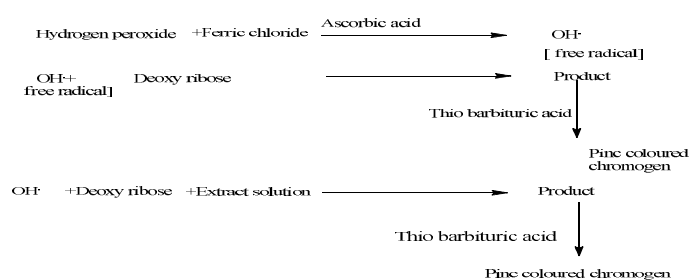
**Anti-Oxidant Studies**

**Chemical Reactions in Nitric Oxide Scavenging Assay**



**Chemical Reactions in Hydroxyl Radical Scavenging Assay**

**Hydroxyl Radical Scavenging Assay**



**Anti Bacterial Activity**

**Disc Difusion Method**

When a filter paper disc impregnated with a chemical is placed on agar, the impregnated chemical will diffuse from the disc into the agar. This diffusion will place the chemical on the agar only around the disc. The solubility of the chemical and itsmolecular size will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the chemical. This area of no growth around the disc is known as a “zone of inhibition”.

**Dispenser method**

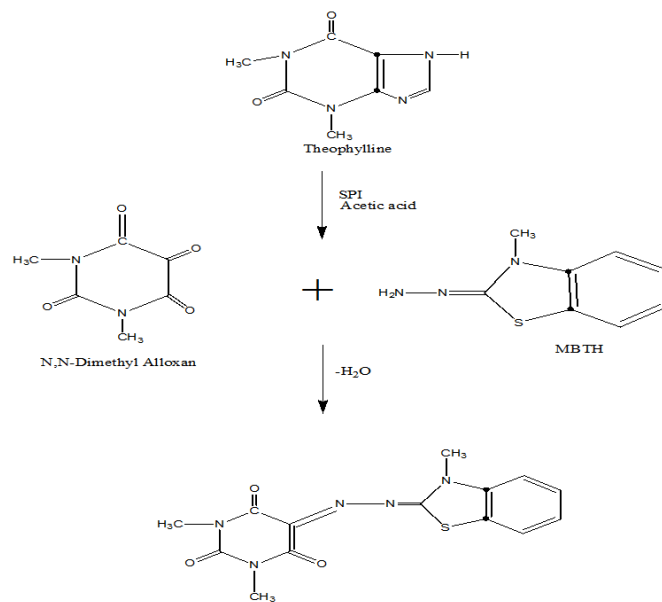
- Dispenser was made containing the correct antibiotic disks for thetest sample of organism.
- It was emulsified using a sterile loop in the sterile saline solution.
- Dispenser was placed over the surface of the plate and used the lever/plunger to dispense the disks.
- The disks used gently pressed onto the surface of the agar, taking care not to press them into the agar.

- The plates were inverted and incubated for 24 hours at 37° C.
- The zone of inhibition (if present) for each antibiotic were measured using metric ruler.
- The measurement obtained from the individual antibiotics were compared to the table of standards to determine if the bacterial species tested was resistant or sensitive to the antibiotic

**RESULTS AND DISCUSSION**

Phyto chemical screening results **Table 1**

	Name of the tests	Result		Inference
		Chloroform extract	Aqueous extract	
1	Morquies test	+	-	Presence of Alkaloids in chloroform extract and absence in aqueous extract
2	Mayers test	+	-	
3	Dragendorffs test	+	-	
4	Hayers test	+	-	Absence of Quinine
5	Wagners test	+	-	
6	Brmine– ammonia test	-	-	Absence of Morphine
7	Iodic acid test	-	-	
8	LeibermannBuchard test	-	-	Absence of Terpenoids
9	Shinoda’s test	+	+	
10	Labat test	-	-	Presence of Flavanoids
11	FeCl <sub>3</sub> test	+	+	
12	Gelatin test	+	+	Absence of Methenedioxy group
				Presence of phenols
				Presence of tannins



**Fig 1** Structure of Alkaloid

**Estimation of Phytocostituents**

**Estimation of Alkaloid by Singh Mbth Method**

**Standard: Thyophylline**

The quantitative determination of alkaloid was carried out by SINGH MBTH method.

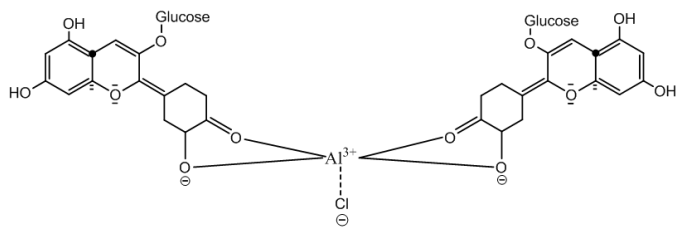


Fig 2 Structure of Flavonoids

In this method, a series of theophylline solution were prepared and after adding the required reagents the OD was measured for each concentration. The concentration of theophylline and corresponding optical density values are showed in figure 3

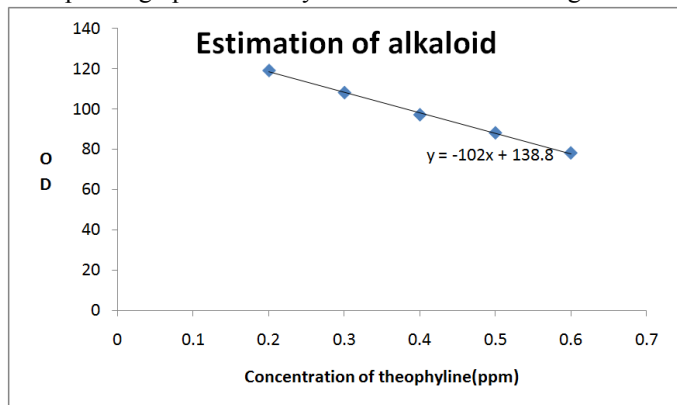


Figure 3

The above calibration graph is best fitted to the equation  $y=102x+138.8$

Using this equation

Percentage of alkaloid in Chloroform extract = 0.4098 ppm

Percentage of alkaloid in aqueous extract = 0.733 ppm

#### Estimation of Flavonoids by Zhishen $AlCl_3$ Method

##### Standard: Rutin

The quantitative determination of Flavonoids was carried out by **ZHISHEN  $AlCl_3$**  method. In this method, a series of Rutin solution were prepared and after adding the required reagents the OD was measured for each concentration. The concentration of Rutin and corresponding optical density values are showed in figure 4.

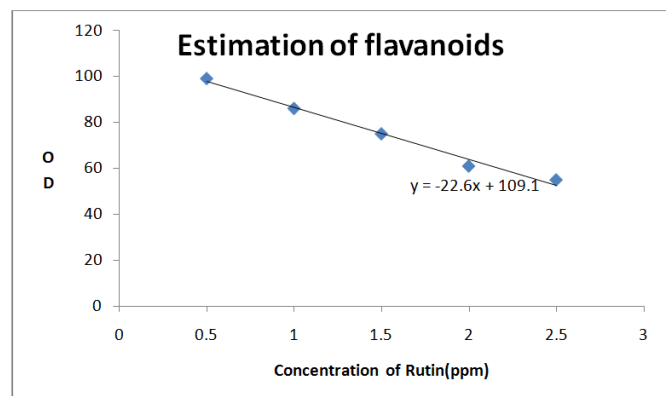


Figure 4

The above calibration graph is best fitted to the equation  $y=22.6x+ 109.1$  from this equation

Using this equation

Percentage of Flavonoids in Chloroform extract = 1.8628 ppm

Percentage of Flavonoids in aqueous extract = 2.5265 ppm

#### Estimation of Total Phenolics By Folin –Ciocalteu Method

##### Standard: Gallic Acid

The quantitative determination of Phenolics was carried out by **Folin –Ciocalteu** method. In this method, a series of Gallic acid solution were prepared and after adding the required reagents the OD was measured for each concentration. The concentration of Gallic acid and corresponding optical density values are showed in figure 5

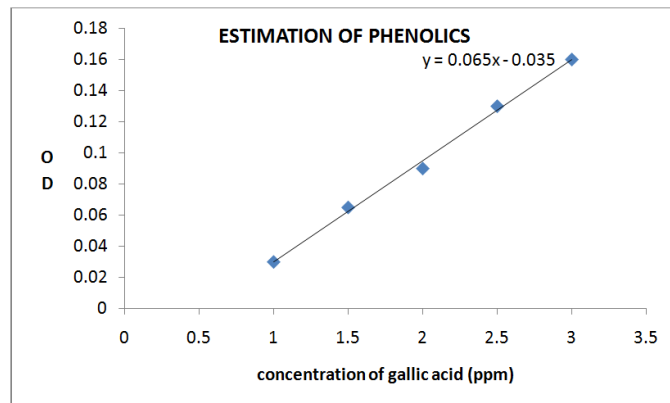


Figure 5

The above calibration graph is best fitted to the equation  $y=0.065x-0.035$

Using this equation

Percentage of Phenolics in Chloroform extract is  $x= 2.5384$  ppm

Percentage of Phenolics in aqueous extract is  $x= 2.6923$  ppm

#### Estimation of Tannins by Prussian blue Method

##### Standard: Tannic Acid

The quantitative determination of Tannins was carried out by Prussian Blue method. In this method, a series of Tannic acid solution were prepared and after adding the required reagents the OD was measured for each concentration. The concentration of Tannic acid and corresponding optical density values are showed in figure 6.

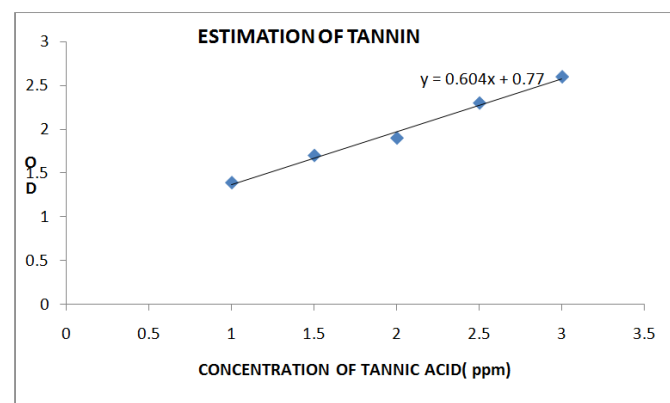


Figure 6

The above calibration graph is best fitted to the equation  $y=0.604x+0.77$

Using this equation

Percentage of Phenolics in Chloroform extract is  $x= 2.5384$  ppm

Percentage of Phenolics in aqueous extract is  $x= 2.6923$  ppm

#### Anti-Oxidant Studies

##### Nitric Oxide Radical Scavenging Assay of Extract

The nitric oxide scavenging assay of chloroform extract and aqueous extract were studied and the results obtained are showed in figure 7.

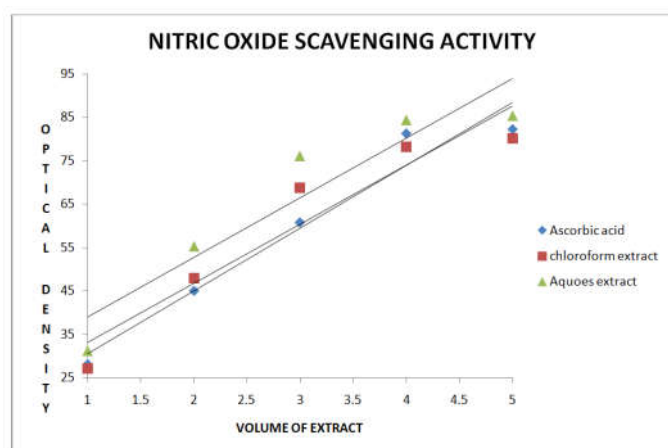


Figure 7

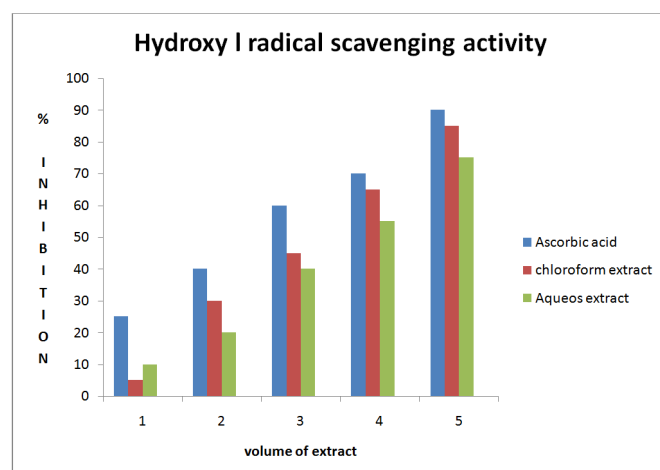


Figure 10

**Calculation of Percentage Inhibition**

Using the OD values the percentage inhibition of standard ascorbic acid chloroform extract, and aqueous extract were calculated. The percentage inhibition results showed in figure 8

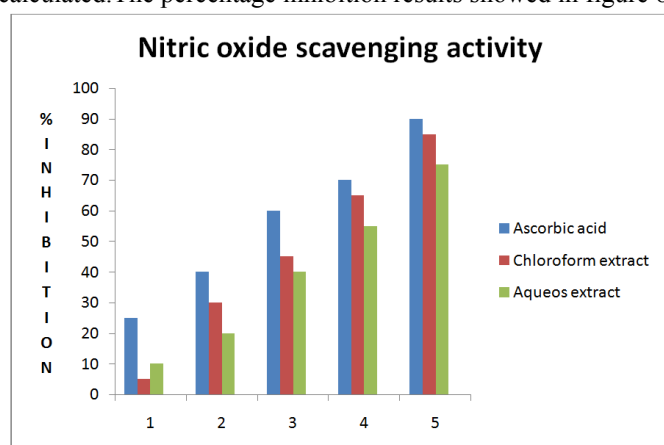


Figure 8

**Hydroxyl Radical Scavenging Assay of Extract**

The hydroxyl radical scavenging assay of chloroform extract and aqueous extract were studied and the results obtained are showed in figure 9

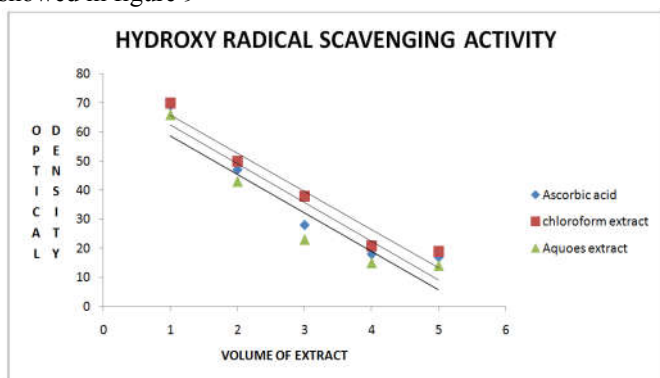


Figure 9

**Calculation of Percentage Inhibition**

Using the OD values the percentage inhibition of standard ascorbic acid chloroform extract, and aqueous extract were calculated showed in figure 10.

**Antibacterial Activity**

The anti bacterial activity of chloroform and aqueous extract were tested for a gram negative bacteria (*E.coli*) and gram positive bacteria (*staphylococcus aureus*), using chloramphenicol as standard by disk diffusion method. The zone of inhibition for *E.coli* bacteria was measured in millimeter for different concentration of the sample.

The zone of the inhibition for various concentration for the sample were tabulated as showed in table 2 and table 3.

**Anti-Bacterial Activity**

**Table 2** Zone of Inhibition Species *E.coli*

	100 mg	200 mg	300mg	500 mg
Chloroform extract	16.63	18.2	19.5	20.5
Aqueous extract	18.4	19.3	20.6	21.7
Standard: chloramphenicol	22.3	25.6	28.2	29.8

**Table 3** Species: *Staphylococcus Aureus*

	100 mg	200 mg	300mg	500 mg
Chloroform extract	6.63	8.2	9.5	11.5
Aqueous extract	8.4	9.3	10.6	11.7
Standard: chloramphenicol	10.2	11.4	12.1	13.5

**SUMMARY AND CONCLUSIONS**

In the present study lemongrass plant was collected, dried and powdered. The powder was subjected to Soxhlet extraction using chloroform and water as solvent. The extracts were concentrated and the constituents were separated by column chromatography using Petroleum ether, 10% Benzene in Petroleum ether, 50% Benzene in Petroleum ether, 100% Benzene, 10% chloroform in Benzene, 50% chloroform in Benzene, and 100%chloroform as eluent. The various fractions were collected and TLC was run with chloroform as developing solvent. The fraction which gave only one spot in TLC test was used to identify the specific constituent. Test for alkaloid, flavonoids, Tannins and phenolic were carried out.. The phyto chemical analysis shows that it contains alkaloid, flavonoids, Tannins and phenolic. The constituents were estimated using colorimetric method. The anti oxidant study of the extracts were carried out by in vitro trials. Nitric oxide free radical scavenging characteristics and hydroxyl radical scavenging activity of the extracts were determined. Their inhibition towards the free radical produced, were determined

using optical density measurements. The values obtained were compared with the standard natural anti oxidant ascorbic acid. Concentration versus inhibition graphs were drawn. The graphs obtained reveal the fact that the anti oxidant property is concentration dependent. The bar diagram shows the quantitative inhibition. Antioxidant effectiveness in vivo depends on the bioavailability of responsible compounds; which was assumed to be low. However, recent studies with improved methodology indicate that some plant phenolics appear in plasma and body tissues and, thus, may be important nutritional antioxidants. It is necessary to conduct clinical trial study, to support the laboratory analysis of having high antioxidant capacity, There are no clinical trials with lemon grass on the Indian population, either on healthy or on diseased populations to support its antioxidant claims or its use in any therapeutic condition. Thus efforts need to be directed to assess the pharmacological potential of lemon grass.

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