Phytochemical Investigation and Development of HPTLC Fingerprint Profile Of Cymbopogon Citratus (Lemongrass).

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Abstract-

Cymbopogon Citratus is commonly known as Lemongrass. This study deals with the moisture, Ethanol Soluble Extractive, water soluble Extractive, Total Ash Value and Acid insoluble ash Value contents were, 4.96%,9.5%,15.4%,96.58%,1.32% respectively. The Phytochemical revealed the presence of carbohydrates, alkaloids, triterpenes, glycosides. The antibacterial activity of Lemongrass leaves was tested against six pathogens and HPTLC Fingerprint.The details are discussed in the paper.

Keywords- Physiochemical, Phytochemical, HPTLC fingerprints, antimicrobial screening.

INTRODUCTION

Medicinal plants had a great importance to the individual health[1].The medicinal value of plant lies in the bioactive constituents of the Medicinal plant[2,3].Phytochemicals were bioactive compounds found in vegetables, fruits, cereal grains and plant-based such as tea and wine. PhytochemicaI consumption are associated with a decrease impact to antioxidant effects[4,5].Plants are very importance sources of drugs used for centuries in the treatment of various microbial infections. Plants based drugs are used for centuries in the treatment of various infections[6]. Plants based drugs are used worldwide for the treatment of diseases because of their easy availability and less toxic effect compared to synthetic drugs. Cymbopogon Citratus is commonly known as Lemongrass.it belongs to the poaceae family.It is a medicinal and aromatic plant. it is an aromatic tall grass with rhizomes[7].

Botanical Classification- [7]

Kingdom-plantae
Sub kingdom - Tracheobionta
Phylum- Magnoliophyta
Class- Liliopsida
Order- poales
Family- poaceae
Genus-**Cymbopogon**
species-**Citratus**

Cymbopogon Citratus are flourishes in sunny, Warm, humid conditions of the tropics and grown in a wide variety of soil ranging from rich loam to poor laterite but calcareous and water logged soils are unsuitable for its cultivation[8].it is locally known by different names such as 'Gawati Chah', Nibugrass, puthiganda etc.The three species of Lemongrass are found in india[9,10]. Many biologically active substances has been isolated and elucidated in cymbopogon citratus. The l important well as relieve spasms, muscle, cramps,rheumatism and head ache[11].Cancer is among the causes of moratality among human population of all ages. it is responsible for 7.6 million death in 2008[12]. The plant is commonly used in folk medicine countries since it exhibit antioxidant properties which in pagation of free radical reaction Present investigation qualitative and quantitative chemical analysis were carried out using Lemongrass grown in kodaikanal[13].

Medicinal uses of Cymbopogon citratus (Lemongrass)-

Lemongrass leaves had been traditionally used to treat various medical and is conditions due to various secondary metabolites present in it.it has been also used to treat fever,cough, elephantiasis,flu,Leprosy, malaria and other digestive problems[7].

MATERIALS AND METHOD-

Sample collection and powder preparation-

Fresh leaves of Lemongrass (Cymbopogon citratus) were collected in March from the **Aarogyadham chitrakoot**. The leaves

were washed under running tap water and then washed with methanol. And dried under shade at room temperature separately. Then was grounded in to powder by using electric grinder and finally the sample was stored in air tight container to prevent moisture and was used for further experimentation.

preparation of sample extracts-

2gm.of powdered sample with 50ml. ethanol and distilled water and kept in a closed iodine flask for 24 hours. Mixture shaked frequently during first 6 hours then the solution was filtered by using whatmann filter paper no.1.both the extracts were used for the phytochemical Analysis.

1-Physiochemical Tests

Determination of* M*oisture content (Loss on Drying at105°C)–

2 gram of powder in two preweytted petridish was taken kept in hot air oven for five hours and then cool in a desiccator for 20 minutes and was weighed.Again,place in oven for half hours and then dessicate for 20 minutes.Calculate the % LOD with reference to air dried powder.

Determination of Ethanol soluble Extractive value-

For ethanol soluble extractive, 2gm. air dried sample was macerated with 50 ml.ethyl alcohol and kept in a closed iodine flask for 24 hours,mixture shaked frequently during first 6 hours and then was allowed to stand for 18 hours. Then the solution was filtered by using Whatmann filter paper no.1 and 10 ml. filterate was evaporated to dryness in a treated flat bottomed petridish over boiled on water bath then dried, cooled. and weighed. Calculated the percentage of ethanol soluble extractive with reference to the air dried sample.

Determination of Water-soluble Extractive value-

Water soluble extractive was determined in the same way as given above instead of alcohol; water was used as solvent. Then calculated the percentage of water-soluble extractive with reference to the air-dried sample.

Determination of Total Ash-

Incinerate about 2gm. of accurately sample in a silica crucible at a temperature up to 500°C until free from carbon. Cooled in a desiccators and Weighed. Then the percentage of total ash was calculated with reference to air dried sample.

Determination of Acid insoluble ash-

Ash was boiled with 25ml. of 5 % Hcl for 5 minutes and was filtered through Whatmann no.42 filter paper and washed the insoluble matter with hot distilled water then, ignited cooled in a desiccator and weighed. The percentage of acid insoluble ash was calculated.

Phytochemical Analysis

Test for Alkaloids -

Mayer's test-

Added few drops of Mayer's reagent to 1ml. of the acidic aqueous extract of sample and the colour was noted.

Dragendroff's test-Dissolved a few mg. of alcoholic or aqueous extract of sample in 5ml.of distilled water, added 2ml HCl until an acid reaction occurs,then added a1ml.of Dragendroff's reagent and observed the colour.

Wagner's test- 2- 3ml.filtrate with few drops Wagner's reagent.and the precipitates were noted.

Hager's test-2-3ml. filtrate was treatedwith few drops of Hager's reagent and was observed the colour.

Test forCarbohydrates a-Fehling's test-Take 2ml.of queous extract of sample was added 1ml. of mixture of equal amounts of Fehling's solution A & B and boil the content of the test tube for few minutes.and observed the precipitate.

Benedict's test-

Mix equal volume of Benedict's reagent and test solution in test tube. Heat on boiling water bath for 5 minutes and observed the colour.

Tannin-Lead acetate test-To the filtrate, a few drops of aqueous basic lead acetate solution was added and observed the precipitate.

Sterols and Triterpenoid-

Salkowski test-

Proteins-Ninhydrin test-The appearance of violet colour indicated the presence of protein. Ninhydrin test about 0. 5mg.of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated and colour was noted.

Test for Coumarins-With ammonia - Take a drop of ammonia on a filter paper, to this add a drop of aqueous extract and observed the Coumarins.

Saponins-Added 2ml.of the aqueous extract of C. Citratus and shaken vigorously with 2 ml. distilled water in a test tube and observed the saponins.

Test for Glycosides-0.5 mg.of leaves extract was dissolved in 1ml.of water and then aqueous NaOH solution and the colour was noted.
Resins-Added 1ml.alcoholic or aqueous extract in 2ml. of acetone and added 1ml of d/w and indicates the resins.
Phenol-1ml. of plant extract, when treated with few drops of $FeCl_3$ solution and the colour was noted.
Test for reducing sugar -To 1ml. of extract added 1ml.of Fehling's A solution and 1 ml of Fehling's B solution and the colour was noted.
High Performance Thin Layer Chromatography (HPTLC)-High Performance Thin Layer Chromatography is an enhanced from of Thin Layer Chromatography (TLC). A number of enhancements can be made to the basic method of Thin -Layer Chromatography.
Preparation of Extract-2 gm.powder of Cymbopogon citratus leaves was defeatted with methanol.On a water bath for 30 minutes. Concentrated under reduced pressure and dried. A stock solution of Cymbopogon citratus leaves methanolic extract was prepared in methanol.

HPTLC Methods-Suitably diluted stock solutions were spotted on precoated silica gel TLC plates with the help of CAMAG LINOMAT V. applicator plates were developed in solvent system of different polarities to resolve polar and non-polar components of the extract. The developed plates were sccaned using TLC scanner 3 (CAMAG). The non-polar components (Steroids and terpenoids) the extract was resolved using a solvent of Toluene: Chloroform: Ethyl Acetate (10:2:1).

4–Antimicrobial Screening–

Media Preparation -To determine be antimicrobial Screening in the six-sample media were prepared.1- 3.75 gm. EMB Agar Base +1.5 gm. Agar powder in 100ml. of distilled water for the growth of E.Coli.2- 4.00 gm. Soyabean Casein Digest Agar +1.5 gm.Agar powder in100ml.of distilled water for the growth of T.B.C.3- 3.90 gm. Potato Dextrose Agar +1.5 gm Agar powder in 100 ml. of distilled water for the growth of Yeast and Mould.- 4.67 gm. Cetrimide Agar Base +1.5gm. powder in 100ml. of distilled water for the growth of pseudomonas.-4.06 gm. Salmonella Agar +1.5gm. Agar powder in100ml. of distilled water for the growth of Salmonella.-4.06 gm. Violet red bile glucose +1.5gm. Agar powder in 100ml. distilled water for the growth of Staphylococcus.In a tube with we took about 15ml.of molten media at a suitable temperature,1ml. of sample was added
then mixed the sample properly in the media and poured the media in to a sterile petriplate.
Now out of these four media will be autoclave and two media of non-autoclave.Closed the lid of the petriplate and allowed the media to completely solidify.Incubated the plate in an inverted position under suitable incubation. condition (mostly for 24 hours at 37°c). And then keep those two non-autoclave pourplates in room temperature for 72 hours.and after then observe and noted the result.

RESULTS AND DISCUSSION

The sample was screened for Moisture Content, Ethanol Soluble Extractive Value, Water Soluble Extractive Value,Total Ash Value, Acid nsoluble Ash and the results are tabulated in table 1and table 2to5 for phytochemical test, HPTLC and Antimicrobial Screening listed inTable 6,7 and8.

Table1: Moisture content105°C (LOD)-

S.NO.	Empty weight of petridish	Empty weight of petridish+ 2.0 gm. sample (M)	Weight after drying		Difference(M-M2)
			(M1)	(M2)	
1.	31.4111	33.4111	33.3330.	33.3061	0.105
2.	31.27464	33.27464	33.19699	33.1812	0.09344

Average=(0.105+0.09344)/2
= 0.0992
LOD=(Averagex100)/weight of sample taken
=(0.0992x100)/2

LOD=4.96%

Table 2:Ethanol Soluble Extractive Value-

S.NO.	Empty weight of petridish (M1)	Add Volume of sample extract.	Weight after evaporation (M2).	Difference-M2-M1).
1.	35.2369	10ml	35.2559	0.019
2.	31.1643	10ml	31.1834	0.0191

Average = (0.019+0.0191)/2
= 0.0190
Ethanol soluble extract=(Averagex500)
=0.0190x500
Ethanol Soluble Extractive value=9.5%
Table3: Water Soluble Extractive Value-

S.NO.	Empty weight of petridish (M1)	Add Volume of sample taken	Weight after evaporation(M2)	Difference(M2-M1)
1.	35.5241	10ml.	35.5546	0.0305
2.	35.8038	10ml.	35.8349	0.0311

Average =(0.0305+0.0311)/2
= 0.0308
Water soluble extract = (averagex500)
=0.0308x500
water soluble exractive value=15.4%
Table 4: Total Ash Value

S. NO.	Weight of Empty Crucible	Empty weight of crucible+ 2gm. Sample (M1)	Weight after incineration	Difference(M1-M2)

			1st Day wt. |2nd day wt. |3rd Day wt.(M2)			
1.	14.9810	16.981	15.0523	15.0505	15.0493	1.9317
2.	17.5033	19.5033	17.5741	17.5730	17.5717	1.9316

Average= (1.9317+1.9316)/2
= 1.9316
Total Ash=(averagex100)/weight of sample taken
= (1.9316x100)/2

Total Ash=96.58%

Table5: Acid Insoluble Ash

S.NO.	Crucible weight(M)	First Day Reading(M1)	Second Day Reading (M2)	Third day Reading	Difference(M2-M)
1.	14.9810	15.0095	15.0091	15.0090	0.028
2.	17.5033	17.5297	17.5286	17.5282	0.0249

Average= (0.028+0.0249)/2
= 0.0264
Acid Insoluble Ash (%) =(averagex100)/weight of sample
= (0.0264x100)/2

Acid Insoluble Ash=1.32%

Table6: Results of Phytochemical test-

S.No.	Phytochemicals	Observation	Results	
			Water Extract	Ethanol Extract
1.	**Alkaloids**			
	a. Mayer's test	White or pale yellow colour was formed	+	-
	b. Dragendraff's test	Orange red colour was appear	+	-
	c.wagner's test	Reddish Brown appeared	-	-
	d.Hager's test	Yellow is colour appear	-	-
2.	**Carbohydrates**			
	a. Benedict test	Green, Yellow or redish appear	+	-
	b.Fehling test	Red brick colour appeared	-	-
3.	**Tannins**			
	a.lead acetate test	Reddish brown colour is appear	+	+
4.	**Steroids and Triterpenoids**			
	a.Salkowski test	Greenish yellow	+	+

		Colour is appear		
5.	**Test for Proteins**			
	a. Ninhydrin test	Violet colour is appear	-	-
6.	**Test for Coumarins**			
	a.with ammonia	Fluorescence	-	+
7.	**Saponin**	Emergence of bubbles	-	+
8.	**Test for Glycosides**	Yellow colour appeared	+	-
9.	**Test for resins**	Taridity appeared	+	-
10.	**Phenol**	Blue Green colour appeared	-	+

(+) Presence; (-) Absence

The Rf values were shown in table 7-

Table-7 Rf Values and Colour

S.No.	RfValues	254 nm Before derivitization	366nm beforederivitization	366nm afterderivitizatio
1.	Rf1	0.25	0.08(Red)	0.08 (Red)
2.	Rf2	0.89	0.24(Red)	0.24 (Red)
3.	Rf3	-	0.30(Red)	0.30(Red)
4.	Rf4	-	0.36(Red)	0.33(Blue)
5.	Rf5	-	0.52 (Red)	0.36 (Red)
6.	Rf6	-	0.74 (Red)	0.56(Light Blue)
7.	Rf7	-	0.82 (Red)	0.67(Sky Blue)
8.	Rf8	-	0.89 (Re	0.74 (Red)
9.	Rf9	-		0.82 (Brown)
10.	Rf10	-		0.89 (Red)

Table-8 Results of Antimicrobial Screening in Cymbopogon citratus-

S.NO.	Antimicrobial Screening	Results
1-	Staphylococcus	Absent
2-	Salmonella	Absent
3-	Pseudomonos	Absent
4-	E-Coli	Absent
5-	Total bacterial counting (T.B.C.)	Present 900
6-	Yeast and Mould	Present 120

DISCUSSION-

Results of the present study reveals the presence of various Phytochemicals i.e.-
Carbohydrates,alkaloids,triterpenes,Glycosides are Present in water extract of the sample, while it was absent in the ethanol extract.Tannins, sterols and triterpenoids, were present in the both the extract that is water and ethanol extract.Coumarins,Saponins and Phenols were present in ethanol extract.HPTLC (HighPerformanceThin Layer Chromatography)-Rf values were taken at 254nm before derivitization in these two spots were observed at 0.25 and 0.89 for Rf and Rf1 sample respectively.At 366nm.before derivitization eight spots were seen i.e., 0.08 (Red), 0:24(Red),0.36 (Red), 0.52 (Red), 0.74 (Red), 0.82 (Red) and 0.89(Red) •At 366nm after derivitization and ten spots were seen i.e., 0.08 (Red), 0.24 (Red), 0.30 (Red),0.33 (Blue), 0.36 (Red), 0.56 (Light blue),0.67(Sky blue),0.74 (Red) ,0.82 (Brown), and 0.89 (Red).

ANTIMICROBIAL SCREENING-Antimicrobial Screening for the sample was done and results (Table-8) reveal the presence of TBC and Yeast and Mould while staphylococcus, salmonella, pseudomonas and E. coli colonies were totally absent.

CONCLUSION

Cymbopogon Citratus has great Medicinal Properties.Ayurvedic herbal medicines ensure physical and mental health without side effects containing the natural ingredients. Ayurvedic herbal medicines help to bring Arogya to human body and mind while allopathic drugs/ medicines have more side effects due to the use of various chemicals and they are harmful and sometimes they are fatal. The preliminary Phytochemical screening and HPTLC fingerprint shows the presence of various Phytochemicals and so it can be used in treating diseases after proper quality evaluation.

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