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Antimicrobial, Antioxidant and Anthelmintic Activities of the Essential Oils and methanol Extract of *Cymbopogon Citratus*

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Abstract: Antibacterial activities of aqueous and methanol extract of *Cymbopogon citratus* leaves were determined against four bacteria by disc diffusion method. Both extracts were found to exhibit inhibition against the isolates. Methanol extract exhibited high inhibitory activity against all the tested bacteria in order of sensitivity as *Staphylococcus aureus* > *Streptococcus bovies* > *Salmonella typhi* > *Escherichia coli*. The antioxidant capacity of the methanol extract and essential oils was determine by H_2O_2 radical scavenging method and the antioxidant capacity of the methanol extract were better than the essential oil. Inhibition of microbial growth was greater in methanol extracts, due to the presence of flavonoids and other non-volatile constituents.

Key words: *Cymbopogon citratus,* antibacterial, Antioxidant capacity.

Introduction

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 phytochemical, including the flavonoids, terpenoids, polyphenolics, carotenoids, coumarins, saponins, component[1], hence promoted as "functional food". The present paper highlights the functional properties of *Cymbopogon citratus*, which has been consumed in various forms such in Thai, Vietnamese and South East Asian cuisines. It is a highly rated folk medicine in Brazil and has been associated with health claims such as treatment in coughs, constipation, elephantiasis flu, headache leprosy, malaria, pneumonia, diarrhea and stomach ache[2]. It has been claimed to be anti-inflammatory, vasorelaxing, diuretic, remedy in treating ringworm infestation, for nervous, gastrointestinal disturbances, fevers and hypertension. *Cymbopogon citratus* has high antioxidant levels. Essential oil extracted from leaves of *Cymbopogon citratus* used as anti-bacterial, and anti-oxidants activities. These EO have the potential to physically stop growth and oxidized .There for the present study will initiated to study anti-bacterial and anti-oxidants activity of essential oil from leaf of *Cymbopogon citratus*.

MATERIALS AND METHODS

Sample collection and preparation

The medicinal plant *Cymbopogon citratus* was selected for this research work due its traditional medicinal and antioxidant activity use among the other plants. The fresh leaves of *Cymbopogon citratus* were collected from Borasolawa southern zone Tigray Region of Northern Ethiopia during the month of January, February and March 2014. The botanical identification of plant sample was carried out by Addis Ababa University biology department national herbarium, Addis Ababa, Ethiopia and voucher specimens were deposited at the institute.

Extraction of the essential oils

About 150 g of the dried leaves of *Cymbopogon citratus* was grounded to powder mechanically using mortar and pestle and hydro-distillation extraction was done using Clevenger's apparatus for 4 hr. The extracted essential oil was dried over anhydrous sodium sulfate and placed in 4 ^{o}c refrigerator for further investigation[3].

Extraction crude oils

Required quantity of powder (100 g) was weighed and transferred to three different Stoppard flasks, and treated with methanol until the powder is fully immersed. The flasks were shaken every hour for the first 6 hour and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extracts were filtered. The extracts were collected and evaporated to dryness by using a vacuum distillation unit. The final residues thus obtained were then subjected to biological activity.

Antimicrobial activity of *Cymbopogon citratus*

The essential oils and solvent extract were individually tested against Gram positive and Gram negative bacteria by the disk diffusion method.

Preparation of test microorganisms

The activity of the essential oils was tested towards 4 different bacteria: Those are *Salmonella typhi* (Gram-negative), *Escherichia coli (Gram*-negative), and *Staphylococcus aureus* (Gram-positive), *Streptococcus bovies (Gram-positive)*. Microorganism strains were employed for determination of antibacterial activity was provided by Department of microbiology laboratory Veterinary College Mekelle University, Ethiopia.

Anthelmintic activity of *Cymbopogon citratus*

Earthworms of approximately same size range of 4 cm were collected from moist garden soil of Mayanisheti, Mekelle, Ethiopia and authenticated by Zoologist of department of biology Mekelle university, Ethiopia as *Lumbricus terrestris* then washed with saline to remove the soil particles, debris and fecal.

The earthworms were divided into four groups having four earthworms. Group one serve as control, receive only normal saline; group two serve as standard, receive standard Albendazol . Group three serve as aqueous extract and group four serve as methanolic extract of different concentration[4]. All the extracts and the standard solution autoclaved in distilled water were freshly prepared before conducting the assay[5]. The concentration of control, standard and extract 25 mg/ml, 50 mg/ml ,75 mg/ml and 100 mg/ml respectively. Observations were made for the time taken until the paralysis as well as death of

an individual worm occurred[6, 7]. The mean time of paralysis and death was recorded in minutes. The paralysis was declared when the worms were not able to move even in normal saline[8]. Death was considered when the worms lost their motility followed with fading away of their body colors[9].

Hydrogen peroxide radical scavenging (antioxidant assay)

The most active principle having antioxidant property found in botanical products are not only vitamins but also chemicals like phenols, polyphenols and flavonoids. Flavonoids are products of plants metabolism and have different phenolic structure[10]. Hydrogen peroxide radical-scavenging activity was determined by the suggested .method[11] with slight modification. Briefly, A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (10 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. Then, the sample mixture was incubated at 37 ^{o}C for 30 min. The absorbance was measured at 230 nm. A hydrogen peroxide radical solution without sample extracts was used as a control. All analyses were run in triplicates.

Phospho molybdenum scavenging assay

The antioxidant activity of fractions was evaluated by phospho molybdenum method[12]**.** An aliquot of 0.1 ml of the sample dissolved di-chloromethane was combined in a vial with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vial was capped and incubated in a water bath at 95 °C for 90 min. After the incubation, samples were cooled to room temperature, and the absorbance of the mixture was measured at 695 nm against a blank. Percent inhibition of phospho molybdenum radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

RESULTS AND DISCUSSION

Hydrogen peroxide radical scavenging activity

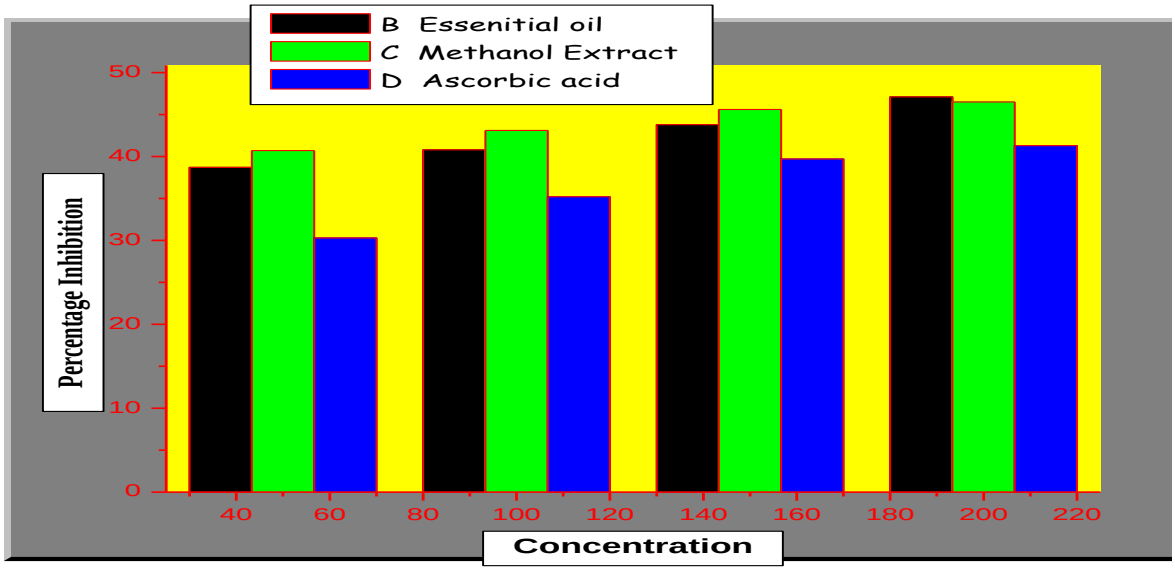

Figure-1 H_2O_2 Antioxidant activities of *cymbopogon citratus* EO

As we see from this bar graph the antioxidants of EOs, MeOH extract and ASA were done in 50,100, 150, and 200 mg/ml concentration of the samples from the Figure-1.The hydrogen peroxide scavenging capacity of the test samples increased with increase in the concentration. The hydrogen peroxide scavenging power of the test samples followed the order of methanol extract > essential oil > ascorbic acid. This showed that methanoic extract of *cymbopogon citratus* had highest hydroxyl radical scavenging effect and was most potent than ascorbic acid standard. Therefore *Cymbopogon citratus* has high antioxidant capacity. .

Phospho molybdenum scavenging assay

The total antioxidant activity of EO and MeOH of *Cymbopogon citratus* by Phospho molybdenum method assay was based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/ Mo (V) complex at acidic pH. From the (Figure-2) one can understand that the MeOH extract has better antioxidant activity than aqueous extract that is 173.02, 350.92, 533.93, 712.36, and 158.99, 319.97, 486.0, 664.81 of grams of Ascorbic acid Equivalent respectively[13]. The possible explanation for methanol extract having high total antioxidant capacity is due to high flavonoids content as flavonoids plays an important role as antioxidants in living systems due to the presence of hydroxyl groups within their structure. The researcher tried to see the antioxidant capacity of some herbs like tomato, Garlic, coriander and Ginger from previous research work[14] they have lower capacity than lemon grass. but we use them in our feeding system so the researcher recommend to do further research works on antioxidant capacity of *Cymbopogon citratus* comparison to the above herbs to incorporates in our diet, because "Supplementation with drugs is never as good as supplementation with foods….".

Experimental approach to study the in-vitro antimicrobial activities

Antibacterial zone inhibition of essential oil and methanol extract were tested on four different bacteria: *Salmonella typhi (*gram-negative*), E. coli* (gram-negative*)* and *Staphylococcus aureus* (Gram-positive) and *Streptococcus bovies* are shown in Table-1 and 2 respectively.

Plant extracts have been used for many thousands of years in food preservation[1]. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of health care. The experimental result showed that the extract screened against two gram-negative bacteria *Escherichia coli*, *Salmonella typhi*, and two gram positive bacteria *Streptococcus bovies* and *Staphylococcus aureus* at four different concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) using disc diffusion method shows that *Cymbopogon citratus* possessed MIC and MBC.

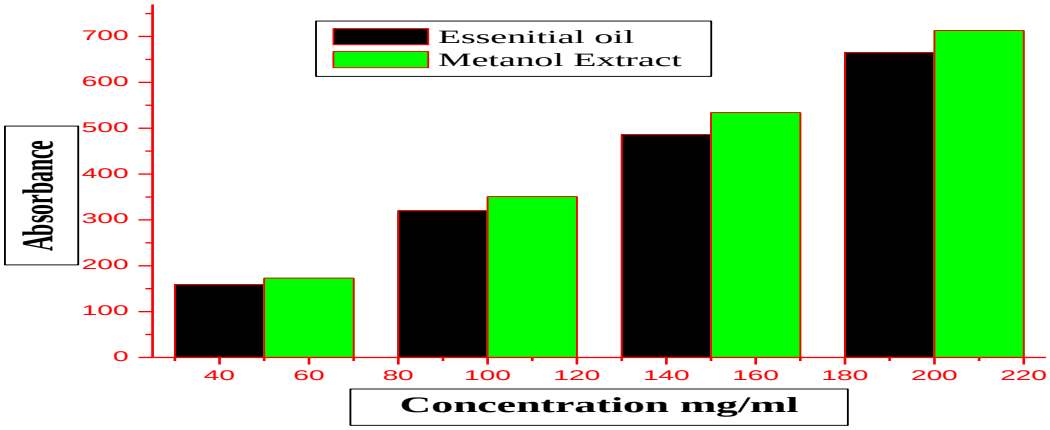

Figure-2 Total antioxidant activities of *Cymbopogon citratus* of EO &MeOH

Table-1: *In vitro* antibacterial activity of water extract at different concentrations

	Tested microorganism	mg/ml	mg/ml	Concentration. of aqueous extract (mg/ml)				Positive Control	Negative Control
		MIC	MBC	25	50	75	100	Amox 30µg	DMSO
1	*Salmonella typhi (ATCC 4028)*	6.25	12.5	8.42± 0.22	9.5± 0.33	10± 0.21	11± 0.21	18.1± 0.01	-
2	*E, coli (ATCC 35218)*	6.25	12.5	8.3± 0.61	9.01 ±0.22	10± 0.01	10.3± 0.10	17.8± 0.25	-
3	*Staphylococcus aureus (ATCC 29523)*	1.56	3.12	10.34± 0.33	11± 0.012	12± 0.011	14± 0.011	19.8± 0.31	-
4	*Streptococcus bovies (ATCC33317)*	3.12	6.25	10.22± 0.01	11.4.±0 .25	12.5± 0.012	13.1± 0.012	18.5± 0.22	-

Table-2: *In vitro* antibacterial activity of methanol extract at different concentrations

	Test microorganism	mg/ml	mg/ml	Concentration. of methanol extract (mg/ml)				Positive Control	Negative Control
		MIC	MBC	25	50	75	100	Amox/30µg	DMSO
1	*Salmonella typhi (ATCC4028)*	3.12	6.25	8.66± 0.55	10.2± 0.021	10.46± 0.55	11± 1.73	17.53± 0.12	_
2	*E, coli (ATCC35218)*	1.56	3.12	7.14± 0.11	8.3±0.1 2	9.5± 1.17	11.4± 0.33	17.12± 0.25	_
3	*Staphylococcus aureus (ATCC29523)*	0.78	1.56	10± 0.01	12.07± 0.11	13.65± 0.10	14.66± 0.557	18.83± 0.31	_
4	*Streptococcus bovies(ATCC 33317)*	0.78	1.56	9.25± 0.11	11.85± 0.11	12.5± 0.012	13.33± 0.22	18.57± 0.49	_

The methanol and aqueous extracts of *Cymbopogon citratus* inhibited the entire test organism, but the percentage inhibition varied with the type of extract concentration, as well as the type of bacteria. Inhibition of microbial growth

was greater at high concentrations of the methanol extracts and, less inhibition was observed as the concentration was lowered.

Though the zone of inhibition was seen in water extract but it was less when compared with methanol extract. This implies that the inhibitory compound of the plant extracts are either more efficacious or exist in higher concentrations and also the inhibitory compound is more soluble in alcohol than water. This shows that the effectiveness of the extracts is directly related to the concentration of the extracts. Several studies have shown that *Cymbopogon citratus* had strong and consistent inhibitory effects against various pathogens[15, 16]. Most studies investigating the action of whole EO, against food spoilage organisms and food borne pathogens agree that, EOs are slightly more active against gram -positive than gram -negative bacteria[17].The gram -negative organisms are less susceptible to the action of antibacterial is perhaps to be expected, since they possess an outer membrane surrounding the cell wall[18], which restricts diffusion of hydro-phobic compounds through its lipo polysaccharide covering[19]. Therefore the plant of this study posses antibacterial activity.

Anthelmintic activity of *Cymbopogon citratus*

From the (Table-3) time required for paralysis and death of *Lumbricus terrestris* was recorded. The time of paralysis and death of the parasite decreases with increment in concentration. This was because of potency of essential oil and methanolic extract. The essential oil demonstrated significant anthelmintic activity against the *Lumbricus terrestris than the* MeOH Extract *Cymbopogon citratus* showed anthelmintic activity .This may be attributed due to alcohol[7, 8].

Table-3 Anthelmintic effect of EO and MeOH

No	Anthelmintic	100 mg/ml		75 mg/ml		50 mg/ml		25 mg/ml		10 mg/ml	
		P min	D min	P Min	D min	P min	D mIn	P min	D Min	P min	D min
1	EO	0.46± 0.21	1.67± 0.11	0.67± 0.2	2.90± 0.11	0.88± 0.13	3.34± 0.22	1.34± 0.13	3.82± 0.33	-	-
2	MeOH	0.59± 0.11	1.92± 0.21	0.78± 0.13	2.96± 0.12	0.94± 0.12	3.36± 0.21	1.44± 0.11	3.65± 0.22	-	-
3	Albendazol	-	-	-	-	-	-	-	-	0.75± 0.1	2.81± 0.1

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