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Evaluation of phytochemicals and antioxidant potential of *Cymbopogon citratus*

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ABSTRACT: *Cymbopogon citratus is an important medicinal plant belongs to family Gramineae. It originates from Ethiopia, India Africa America and is broadly extended all over humid, subtropical and warm temperate regions of the world. This present research work has been designed to evaluate the antioxidant potential of Cymbopogon leaves. The antifungal and antioxidant components of Cymbopogon leaves were extracted by means of four solvent systems (80% methanol, 100% methanol 80% ethanol, and 100% ethanol) and maximum extract yield (48.1 g/100g DW) was obtained in 80% methanolic solvent system. Cymbopogon leaves were analyzed in terms of total phenolic contents, showed that 80% methanolic leaves extract offered highest total phenolic contents (35.2 mg GAE/g DW) Antioxidant activity was investigated by DPPH radical scavenging activity and by measuring reducing power. Results showed that 80% methanolic leaf extract showed maximum radical scavenging activity and reducing potential. Overall results of the present study showed that 80% methanolic Cymbopogon leaf extract can be used effectively to make antioxidant agents which can be utilized in different industries like pharmaceutical, food and cosmetics.*

Keywords: Antioxidant, *Cymbopogon,* Phenolics, Reducing Potential, Lemon Grass

INTRODUCTION

The aromatic plant Lemon grass belongs to the family Gramineae (Akhila., 2010). The leaf-blade of this plant is linear, elongated at both ends and it can raise to a 1.5 cm in width and 50 cm in length. The tubular shape of the leaf sheath acts as a pseudo stem. This plant at mature stage of growth produces flowers (Tajidin et al, 2012). Lemon grass or Cymbopogon citratus (C. citratus widely cultured in warm, tropical and subtropical regions. On dry basis it has 1% to 2% essential oil and its chemical composition may be different as a genetic diversity function, habitat and cultural agronomic treatment (Hadjilouka, 2012).

The important phytoconstituents of lemon grass are essential oils which includes Citral α, Citronellal, phenolic and flavonoids compounds (Vanisha et al.,2012).

Lemongrasses possess antioxidant, bactericidal, antidepressant, astringent, sedative, fungicidal, nervine and antiseptic properties (Naik et al., 2010). According to biological effects C. citratus extracts describe to its main bioactive elements, resulting from its stem, roots and leaves, in addition to secondary metabolites of these compounds (Christopher et al., 2014). The biologically active citral component of lemon grass consists of its essential oil (Huynh et al., 2008).

The current research work was performed to evaluate the biological potential of lemon grass. The phytochemicals and the antioxidant activity were evaluated for C. citratus.

Materials and methods

Collection of plant materials

C. citratus leaves were obtained from the vicinity of Lahore Garrison University, Lahore, Pakistan.

Pretreatment of plant materials

The leaves of C. citratus were washed with tap water and then dried out at 41oC in an oven (Memmert, Jarmany) until stable weight. By using a commercial blender, dried leaves were grounded into fine powder. Then the ground material was conceded through 79-mesh strainer. The passed material was also used for extraction purposes. Polythene bags were used to store the ground samples at 4oC till further analysis.

Extraction of bioactive compounds

For extraction, four solvent systems (100% ethanol and 80% ethanol,100% methanol, 80% methanol,) are being used. In this regard powdered leaves (20g) were also extracted with 200mL in an orbital shaker for 6 hours at room

temperature (Gallenkamp, UK). To separate the extract from residue Whatman No. 1 filter paper was used. Two times resulting residues were extracted with the same solvent system. Drying of extracts was done at temperature of 45˚C and their yield was calculated by weighing extracts. The extracts were reserved in a refrigerator at 4˚C for further analysis (Hassan et al., 2016).

Phytochemical studies of medicinal plant extract

Total phenolic contents

The method which was used to establish the total phenolic contents of C. citratus was based on the procedure of Zafar et al., (2016). The results were presented in gallic acid equivalent (GAE) per gram of extract.

Antioxidant potential

DPPH radical scavenging assay

DDPH radical scavenging analyzes was applied to determine the free radical scavenging activity of C. citratus. 2, 2-diphenyl-1-picrylhydrazyl radical was used to determine the scavenging action as described by Suleman et al., (2018) with little amendment.

Determination of reducing power

The reducing power of the leaf extracts was resolute according to the procedure explained by (Hassan et al., 2016) with slight modification.

Statistical analysis

By performing all experiments in triplicate (n=3), mean ± SD was applied. Data analysed at 5% significant level through statistical software Minitab 2000 Version 13.2 (Minitab Inc. Pennsylvania, U.S.A).

RESULTS AND DISCUSSION

The current research work was conducted to display the phytochemical constituents and antioxidant potential of C. citratus leaves.

Percentage yield (g/100g DW) of extracts

The percentage yield of plant extracts is based on different factors like amount of solvent used, nature of plant material and method of extraction (Hsu and Coupar, 2006). Methanol is known to be a superior and broadly used solvent to pull out antimicrobial components and natural antioxidative components from plants (Anwar et al., 2010).

The extraction yields from leaves of C. citratus against different solvent systems are presented in Table 1. Comparatively, 80% methanol showed significantly ($p<0.05$) higher extraction yields from leaves (48.1%). The extraction capability of different solvent systems from leaves followed the order: 80%

methanol> 80% ethanol> absolute methanol> absolute ethanol.

Table 1: Percentage yield (g/100g DW) of extracts of Cymbopogon leaves

Sr. no	Solvent System	Percentage yield (g/100g DW)
1	80 % Methanol	48.1±0.42[a]
2	80 % Ethanol	45.7±0.23 [b]
3	Absolute Methanol	41.3±0.40 [c]
4	Absolute Ethanol	39.2±0.25 [d]

Values mean ± SD of three samples analysed individually in triplicate at p <0.05. Superscripts alphabets within the column depicted significant differences among different solvent systems.

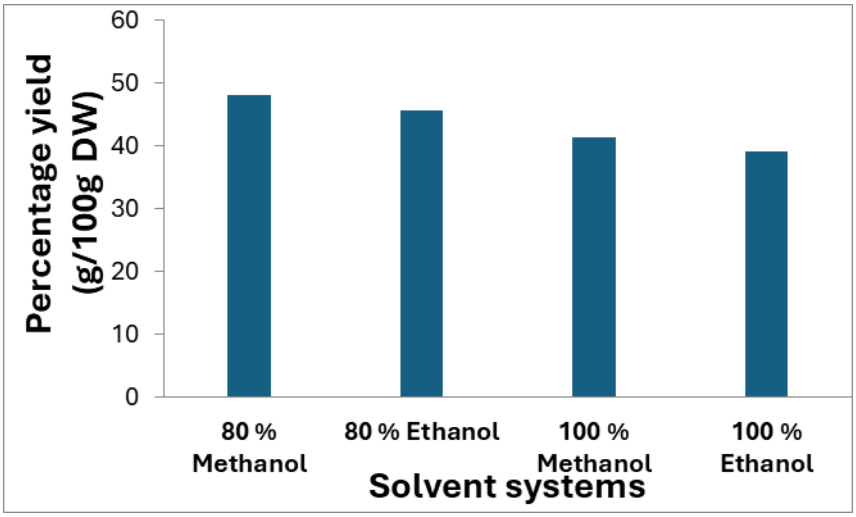

Fig. 1: Percentage yield (g/100g DW) of extracts of *C. citratus* leaves

Total phenolic content

In plants, phenolic compounds are the most important antioxidants (Sakihama et al., 2002). They contribute an essential role in scavenging free radical action (Agbo et al., 2015). Scavenging activity of the potent free radical of phenolic compounds is due to the presence of their hydroxyl groups and it directly contributes to antioxidant potential (Wojdylo et al. 2007). Results of phenolic contents medicinal plant extract are given in Table 2 showed total phenolic content in leaves of cymbopogon was found to be

varied significantly from 29.3-35.2 (mg GAE/g DW), respectively. It was investigated that maximum TPC were obtained from leaves in 80% methanolic extract followed by: absolute methanol>80% ethanol>absolute ethanol. Overall, results showed that the maximum phenolic contents were obtained from methanolic leaf extracts.

Our results are supported by the previous study of Butsat and Siriamornpun (2016) who reported that higher phenolic contents were obtained from 80% methanol, than 80% ethanol for plant extracts. Ghasemzadeh et al. (2011) reported that methanol solvent was found to be more effective in extracting phenolic components as compared to other solvents that also support our results.

Liao et al. (2012) also determined the phenolic contents in different parts of Cymbopogon leaves of the methanol extracts. The maximum phenolic content was obtained from Cymbopogon leaves. Namvar et al. (2017) examined that 80% methanolic extract was found to possess higher total phenolic contents than other extracts. Thus, for extraction of phenolic compounds, an aqueous methanolic solvent system is being used that is a better and more efficient solvent.

Table 2: Total phenolic contents (mg GAE/g DW) of Cymbopogon leaves

Sr. no	Solvent System	Total phenolic contents (mg GAE/g DW)
1	80 %Methanol	35.2±0.56 [a]
2	80 % Ethanol	30.9±0.58 [b]
3	absolute Methanol	32.1±0.43 [ab]
4	absolute Ethanol	29.3±0.35 [c]

Values mean ± SD of three samples investigated individually in triplicate at $p < 0.05$. Superscripts alphabets within the column showed significant difference among different solvent systems

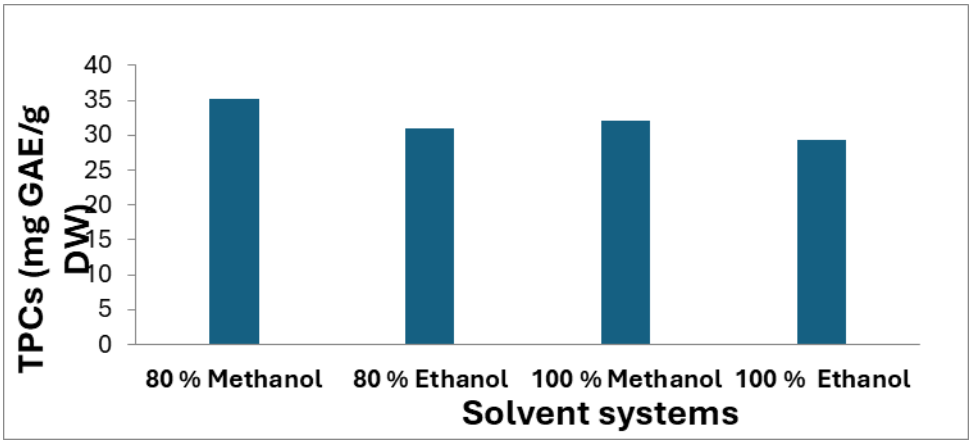

Fig. 2: Total phenolic contents (mg GAE/g DW) of Cymbopogon leaves

Antioxidant activity

There are various numbers of medicinal plants that are being used due to their antioxidant properties. These chemical constituents are very helpful to prevent the destructive actions caused by their oxidative stress (Selvam et al., 2012). In the present study, two assays were used to find the antioxidant activity of Cymbopogon leaves.

DPPH radical scavenging activity

Natural constituents such as polyphenols, flavonoids, phenolics, terpenes and tannins possess antioxidant property to scavenge free radicals (Hassan et al., 2009). Antioxidant activity of these products can be evaluated by using DPPH radical scavenging assay. This assay has been used widely to test the scavenging capability of compounds which act as free radical or hydrogen donors to DPPH (Patel et al., 2015). A nitrogen centred free radical compound is present in stable form of DPPH. Upon reduction, its colour changes from violet to yellow by hydrogen or electron donation. Substances which can execute such types of reactions are known as good antioxidants and better radical scavengers. It has been also found that with increasing the extract concentration, DDPH free radical scavenging ability also increases (Ebrahimzadeh et al., 2010).

So, DPPH free radical scavenging method is based on phenomenon of transfer of electron. It is an antioxidant assay that produces a violet coloration in methanol solution (Garcia et al.,

2012) It is an important mechanism that explains the oxidation process of proton radical scavenger. By decreasing the absorbance of DPPH solution to 517 nm, its reduction capability was evaluated suggesting that antioxidant activity of plant extract is owing to its proton donating capability (Chougule et al., 2012). The antioxidant molecule has the hydrogen donating atom which contributes to its free radical scavenging nature which is an important quality of antioxidants (Sathisha, 2011). DPPH radical assay has been used because it is a quick, reliable, easy and rapid method to investigate the general antioxidant activity of plants extracts as well as pure compounds. This method is also used for showing a lot of samples for radical scavenging potential and is self-governing on the polarity of sample (Aliyu et al., 2009).

The antioxidant potential of medicinal plant C. citratus was evaluated by using the DPPH free radical scavenging assay. This assay also explored its new potential sources for natural antioxidants. DPPH concentrations of medicinal plant leaves were tested and found to be reduced due to scavenging potential. Table 3 showed that there is a significant difference of DPPH radical scavenging activities of C. citratus extracts among different solvent system. The aquoues alcoholic extracts of C. citratus leaves exhibited satisfactory DPPH radical scavenging ability. The C. citratus leaves exhibited highest DPPH radical scavenging potential significantly ($p < 0.05$) in 80% methanolic extract followed by 80% ethanol, absolute methanol and absolute ethanol.

Present results are supported by the preceding report of Liao et al., (2012) who investigated that Cymbopogon leaves exhibit higher free radical scavenging activity. Wu et al., also (2009) reported that methanolic extracts of cymbopogon also showed highest DDPH radical scavenging ability (71.1%).

Table 3: DPPH radical scavenging activity of the *C. citratus* leaves

Sr. no	Solvent System	DPPH (%) radical scavenging activity
1	80 % Methanol	60.1±1.20 [a]
2	80 % Ethanol	58.9±0.45 [b]
3	100 % Methanol	57.3±2.48 [bc]
4	100 % Ethanol	56.4±0.65 [c]

Values mean ± SD of three samples investigated individually in triplicate at p <0.05. Superscripts alphabets within the column showed significant differences among different solvent systems. Reductive abilities of the plant extracts can be an indication of their potential towards antioxidant activities (Zhang et al., 2011). The antioxidant ability of phenolic compounds is generally due to their redox properties. These properties allow them to react as a reducing agent such as an oxygen quencher electron donor. Studies on medicinal plants and vegetables revealed that plants are the great source of antioxidant properties. In biological systems, these plants can apply the protection effects against certain oxidative stress (Sylvie et al., 2014). Electrons are donated to reactive radical species due to the presence of antioxidant substances by the process in which these are neutralized into stable and nonreactive species (Nishaa et al., 2012).

In this assay, lessening of the Fe3+ to the ferrous form occurs due to presence of reducers which is also known as antioxidant. So reducing power is measured by donation of electron and reduction of Fe3+(CN−)6 to Fe2+(CN−)6. Perl Prussian blue colour product formation indicates the presence of Fe2+ concentration that can be monitored at the wavelength of 700 nm (Ahmed et al., 2015). Higher absorbance values were indication of high antioxidant properties (Nishaa et al., 2012). Hence, activity of reducing power increases with increasing the concentration of extracts (Senguttuvan et al., 2014).

The reducing power of C. citratus leaves extracts is offered in table 2. The reducing potential values of the examined extracts were observed at different concentrations ranging from 2.5 to 10.0 mg/mL. It was experimental

that leaves extracts showed considerably (P< 0.05) high reducing potential, irrespective to which type of solvent used. However, 80% methanolic leaves extract showed the highest reducing power. The results revealed that antioxidant power was a function of concentration. By increasing the extract concentration, antioxidant activity was also increased. Results of present research work are supported by the previous analysis of Geng et al., (2015) who investigated that the reducing power of C. citratus leaves linearly increased with increasing the extract concentration. Furthermore, Chang et al. (2007) described the reducing power of C. citratus extracts which also showed greatest reduction potential at 2.5mg/mL concentration.

Table 3: Reducing Power of Cymbopogon leaves extract

Plant Parts	**Solvent system**	**Concentration (mg/ml)**			
		2.5[a]	**5.0[b]**	**7.5[c]**	**10[d]**
Leaves[a]	**80% methanol**	**0.131±0.03**	**0.137±0.02**	**0.145±0.01**	**0.158±0.06**
	80% ethanol	**0.128±0.01**	**o.131±0.04**	**0.145±0.03**	**0.150±0.05**
	Absolute Methanol	**0.121±0.06**	**0.127±0.02**	**0.134±0.01**	**0.151±0.06**
	Absolute Ethanol	**0.127±0.04**	**0.132±0.01**	**0.138±0.06**	**0.145±0.02**

Values mean ± SD of three samples investigated individually in triplicate at p <0.05. The superscripts alphabets within the column showed significant differences among different plant parts. Superscripts alphabets within the rows depicted significant differences among different concentrations.

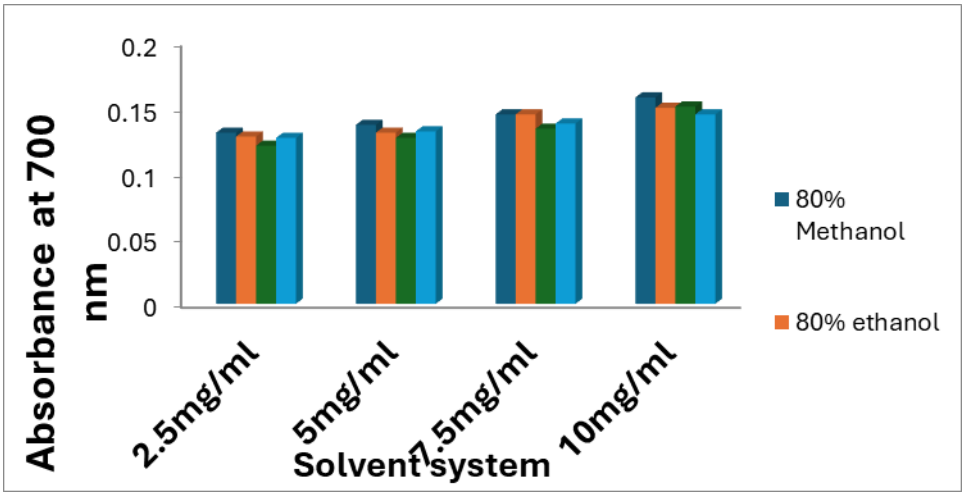

Fig. 3: Reducing Power of *C. citratus* leaf extracts

CONCLUSION

The present research work was conducted to investigate the biological activities of leaf extracts of C. citratus. A considerable quantity of total phenolics was found in examined leaf extracts of C. citratus. It was revealed that leaf extracts of C. citratus exhibited excellent antioxidant activity. Phytoconstituents of plants can be used in food and cosmetic industries to stop the process of oxidation.

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CONFLICT OF INTEREST

Authors declare there is no conflict of interest.

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