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Research paper

Comparative phytochemical screening and antioxidant activity of lemon grass and sweet wormwood leaves extract

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Abstract

Sweet wormwood (Artemisia annua) and lemongrass (Cymbopogon citratus) leaves were extracted with methanol, evaporated to dryness and processed for antioxidant activity and phytochemical screening. Phosphomolybdenum Reagent was prepared using standard procedure. An aliquot mixture of 2mL of extract solution (1000µg/mL, 500µg/mL, 250µg/mL, 125µg/mL, 62.5µg/mL, 31.25µg/mL and 15.625µg/mL) was mixed with 1.8mL of reagent solution. The samples tubes were incubated at 95°C for 90 minutes. The absorbance of the aqueous solutions was measured at 695nm against blank. Antioxidant activities of the plants extract were also measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method. Absorbance was recorded at 517 nm against blank using a UV-Vis spectrophotometer. The result of in vitro antioxidant potential of the methanolic leaves extract showed more increase in concentration the higher the absorbance. Qualitative Phytochemical Analysis of Lemon Grass and Sweet wormwood shows the presence of phytochemical in lemongrass and sweet wormwood such as flavonoid, tannin, steroid, saponins and cardiac glycoside.

Keywords: *Antioxidant; Free Radical; Phytochemical; Lemon Grass; Sweet Wormwood.*

1. Introduction

Phytochemicals are bioactive compounds found in vegetables, fruits, cereal grains, and plant based beverages such as tea and wine. Phytochemical consumption is associated with a decrease in risk of several types of chronic diseases due to their impact to antioxidant and free radical scavenging effects (Yu-Jie et al., 2013). Free radicals are the natural by-product of biochemical processes, that is metabolism in a cell, and when build up, they harm the cells of the body if not scavenged. Yet free radical is essential to life, the body's ability to turn oxygen and food depends on a chain of free radicals. They are also crucial part of immune system, floating through the veins and attacking foreign invaders (László et al., 2005). Cancer and atherosclerosis, two major causes of death, are salient "free radical" diseases. Cancer initiation and promotion is associated with chromosomal defects and oncogenes activation. It is possible that endogenous free radical reactions, like those initiated by ionizing radiation, may result in tumor formation (Lobo et al., 2010). DNA is the major target of free radical damage. The type of damage is many and include strand breaks, various forms of base damages can result in mutations that are heritable change in DNA that can yield cancer in somatic cells or fetal malformations in the germ cells, the role of free radicals with tumor suppressor gene and oncogenes suggest their involvement in developing different human cancer (Miral and Pawel, 2012). Antioxidants are used to counterbalance the effect of free radicals. An antioxidant is a stable molecule which donates an electron to a charged free radical and terminates the chain reaction before vital molecules are damaged, Free radical scavenging property of antioxidants delays or inhibits cellular damage (Satish and Dilipkumar, 2015). Antioxidants are in high need, as they help in lowering ageing signs. Therefore the use of plant products has been increasing every day to lower side effects (Lobo et al., 2010). In recent years, antioxidants have gained a lot of importance due to their potentials as prophylactic and therapeutic agents in many diseases. Traditionally, herbal medicines with antioxidant properties have been used for various purposes, and epidemiological data also point to widespread acceptance and use of these agents (Michael, 2012). Antioxidants can be classified into two major groups; enzymatic and non-enzymatic antioxidants. Some of these antioxidants are endogenously produced, including enzymes, low molecular weight molecules, and enzyme cofactors. Many non-enzymatic antioxidants are obtained from dietary sources. Dietary antioxidants can be classified into various classes, of which polyphenols is the largest class. Polyphenols consist of phenolic acids and flavonoids. The other classes of dietary antioxidants include vitamins, carotenoids, organosulfural compounds, and minerals (Michael, 2012). The objective of this research is to determine and compare the phytochemicals content and the in vitro antioxidant properties of the leaf extract of lemongrass and sweet wormwood.

2. Methodology

2.1. Sample collection

Sweet wormwood (Artemisia annua) and lemongrass (Cymbopogon citratus) were purchased from Wudil market, Wudil L.G.A Kano state. The leaves of the plants were taken, air-dried and pulverized via mortar and pestle into fine powder.

2.2. Plant extraction

The powdered form of lemongrass and sweet wormwood were measured (100g) in two separate flasks and were prepared by soaking in 400ml of methanol each. The two mixtures were left to stand at room temperature for 48 hours and then filtered using Whitman filter paper No. 42 using vacuum pump. The alcoholic filtrates (methanol) were evaporated. All the samples were extracted that is, 50% Methanol was evaporated to dryness and processed for antioxidant activity and phytochemical analysis (Azwanida, 2015).

2.3. Reagents preparation

2.3.1. Phosphomolybdenum reagent preparation

To prepare Phosphomolybdnate reagent solution, (0.6M sulphuric acid, 30mM sodium phosphate and 4mM ammonium molybdate). 3.33ml of sulphuric acid was diluted with 100ml of distilled water (D.H_2O), then followed by adding 0.4 grams of Sodium Phosphate and 0.5 grams of Ammonium Molybdate (Prieto et al., 1999).

2.4. Antioxidant

An aliquot mixture of 2mL of extract solution (1000µg/mL, 500µg/mL, 250µg/mL, 125µg/mL, 62.5µg/mL, 31.25µg/mL and 15.625µg/mL) was mixed with 1.8mL of mixture reagent solution (0.6M sulphuric acid, 30mM sodium phosphate and 4mM ammonium molybdate). The samples tubes were sealed and incubated in a boiling water bath at 95°C for 90 minutes. After incubation, the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank. A typical blank contained 1mL reagent solution and appropriate volume of same volume of the solvent used for the samples and it was incubated under the same conditions as the rest of the samples (Prieto et al., 1999).

2.4.1. DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity

Antioxidant activity of sweet wormwood and lemongrass extract were measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method. The reaction was monitored as a color change from purple to pale yellow. A quantity 0.1ml of sweet wormwood and lemongrass each extract was added to 0.2ml of 100µM DPPH solution in methanol and the reaction mixture was kept in the dark for 45min. Absorbance was recorded at 517 nm against blank using a UV-Vis spectrophotometer. Ascorbic acid was used as standard (Zhu et al., 2006). The radical scavenging activity on DPPH was expressed as,

$$\text{Scavenging activity (inhibition)}\% = \left[100 - \left\{\left(A_s - {}^{A_b}/_{A_c}\right)\right\}\right] X100$$

Where; A_c is the absorbance of control,
A_s is the absorbance of sample extract or standard
A_b is the absorbance of blank.

2.5. Qualitative determination of some phytochemicals

2.5.1. Test for flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

2.5.2. Test for alkaloids

Dragendroff's Test: Extracts were treated with few drops of Dragendroff's reagent. A reddish brown precipitate indicates the presence of alkaloids Wagener's Test: Extracts were treated with few drops of Wagner's reagent. A whitish precipitate indicates the presence of alkaloid.

2.5.3. Test for steroid

Liebermann-Burchard's test: To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently, 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change was observed immediately and later indicates the presence of steroid. A blue or blue green indicates steroids.

2.5.4. Test for cardiac glycoside

Kella-killiani's test: A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. The mixture was observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides.

2.5.5. Test for tannins

Ferric Chloride test: To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate.

2.5.6. Test for anthraquinones

Bontrager's test: To portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones.

2.5.7. Test for saponin frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The test tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb forth persists for 10-15 minutes indicates presence of saponin.

2.5.8. Test for carbohydrates molish's test

To 1ml of the filtrate, 1ml of molish's reagent was added in a test tube, followed by 1ml of concentrated sulphuric acid down the test tube to form a layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate.

3. Result and discussion

3.1. Result

The result of in vitro antioxidant potential (Phosphomolybdenum and DPPH free radical scavenging activity) of the methanolic leaves extracts of lemongrass and sweet wormwood are presented in Fig. 1and 2 respectively. Whereas, the qualitative phytochemical analysis of the above mentioned plant leaves extracts are presented in table 3.

Table 1: Phosphomolybdenum Assay

Concentration (µg/ml)	Absorbance for Ascorbic acid	Absorbance for lemongrass	Absorbance for Artemisia annua
1000	1.0641	16.93	13.2
500	0.5997	18.81	14.66
250	0.3678	15.39	13.2
125	0.2311	12.09	9.429
62.5	0.1380	13.03	11.01
31.125	0.0999	8.465	6.6
15.625	0.0698	1.693	1.32

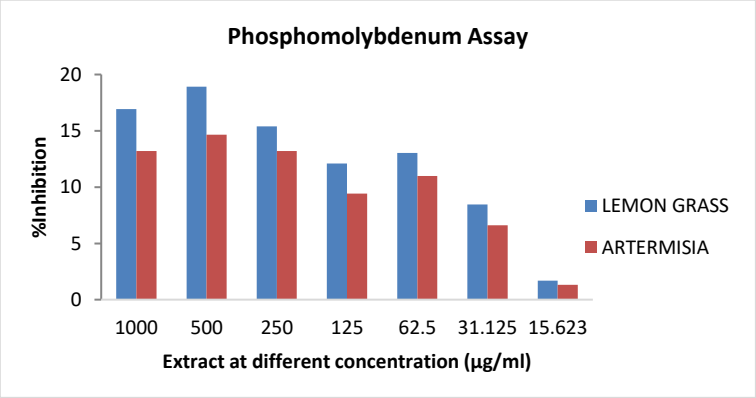

Fig. 1: Inhibition of Phosphomolybdenum in Leaf Extract of Lemongrass and Sweet Wormwood at Different Concentration.

Lemongrass and sweet wormwood show increase in concentration of phosphomolybdenum at increased absorbance. That is, the higher the absorbance, the higher the concentration of phosphomolybdenum. Ascorbic acid was used as standard.

Table 2: DPPH (1,1-Diphenyl-2-Picrylhydrazyl) Assay

Concentration (µg/ml)	Absorbance for Lemongrass	Absorbance for Artemisia annua	Absorbance for Ascorbic acid
1000	81.4392	83.2267	97.94
500	69.5665	70.9062	97.53
250	55.2168	52.3241	96.74
125	37.5444	27.3348	96.57
62.5	25.0142	23.1947	95.74
31.125	19.4812	17.9353	82.28
15.625	17.0398	11.2011	74.11

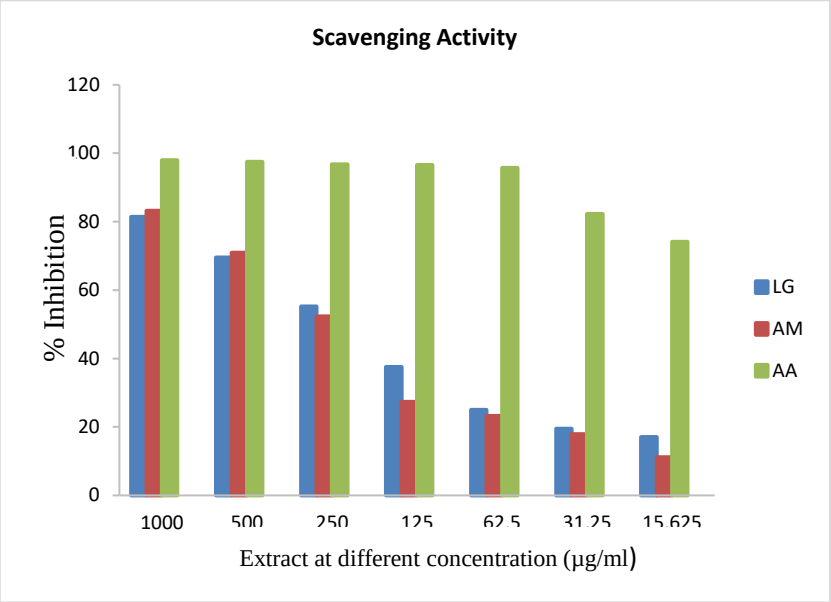

Fig. 2: DPPH Radical Scavenging Activity at Different Concentration of Leaf Extracts of Lemongrass and Sweet Wormwood.

Sweet wormwood and lemongrass have indicated increase in scavenging radical activity when the absorbance increase at all the concentration.

3.2. Phytochemical component

Table 3: Qualitative Phytochemical Analysis of Lemon Grass and Sweet Wormwood

Component	Lemon grass	Sweet wormwood
Alkaloids	-	-
Flavonoids	+	+
Tannin	+	+
Cardiac glycoside	+	+
Anthraquinones	-	-
Carbohydrate	+	+
Steroids	+	+
Saponins	+	+

Key; (+) stand for present and (-) stand for absent.

The result obtained showed the presence of phytochemicals in lemongrass and sweet wormwood such as flavonoid, tannin, steroid, saponins, carbohydrates and cardiac glycoside. However, Anthraquinones and Alkaloids were not found in lemon grass and sweet wormwood.

3.3. Discussion

The results of the qualitative phytochemical analysis in lemon grass leaves and sweet wormwood leaves showed carbohydrate, flavonoid, saponins, cardiac glycoside, tannin and steroid were found present. However, anthraquinones and alkaloids were not found in both lemon grass and sweet wormwood. These secondary metabolites were reported to have many biological and therapeutic properties (Vishnu et al., 2013).

The antioxidant activity was determined to be effective through the various assays. The free radical scavenging activity with respect to DPPH(1,1-diphenyl-2-picrylhydrazyl) showed that lemongrass and sweet wormwood indicated increase in scavenging radical activity at increased concentrations. But at 125µg/ml and 15.625µg/ml concentration reveal that lemongrass exhibit a higher percentage of inhibition of oxidation than the other specie analyzed. Which also means that lemongrass indicated higher scavenging activity compared to sweet wormwood.

The Higher the DPPH radical scavenging activity could be attributed to higher flavonoids and phenolic content in the samples. Base on the experiment observed, lemongrass has higher content of phenol and flavonoid, and these phytochemicals are responsible for the higher DPPH scavenging activity (Jamuna et al., 2014).

The total antioxidant assay shows the ability of reducing the power of phosphomolybdenum in the leaves extracts at different concentrations. These phosphomolybdenum assay result showed that there was an increase in the absorbance when the concentration increases which was determined in the standard calibration curve.

Lemongrass leaves and Sweet wormwood leaves extract showed an increasing order of magnitude; still the difference in the free radical activity is marginal with reference to increase in concentrations of the plant extract tested. However, a correlative relationship has been reported between the phytochemicals such as tannins, phenol and flavonoids and the free radical scavenging activity and antibacterial activity (Kaur et al., 2010). Tannins and flavonoids have therapeutic uses due to their anti-inflammatory, antifungal, antioxidant and healing properties (Thiago et al., 2008). The higher the inhibition the higher the antioxidant activity.

Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species (ROS), which are the harmful by-products generated during normal cell aerobic respiration (Sulekha et al., 2009).

3.4. Conclusion

The findings of the current study have shown that Lemon grass leaves possesses higher Total Phenolic and flavonoid Content than Sweet wormwood leaves which lead to high scavenging activity. However, Lemon grass leaves has expressed higher free radical scavenging effect (i.e., the primary antioxidant activity) as compared to Sweet wormwood leaves.

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