

Report

Kaksekar project



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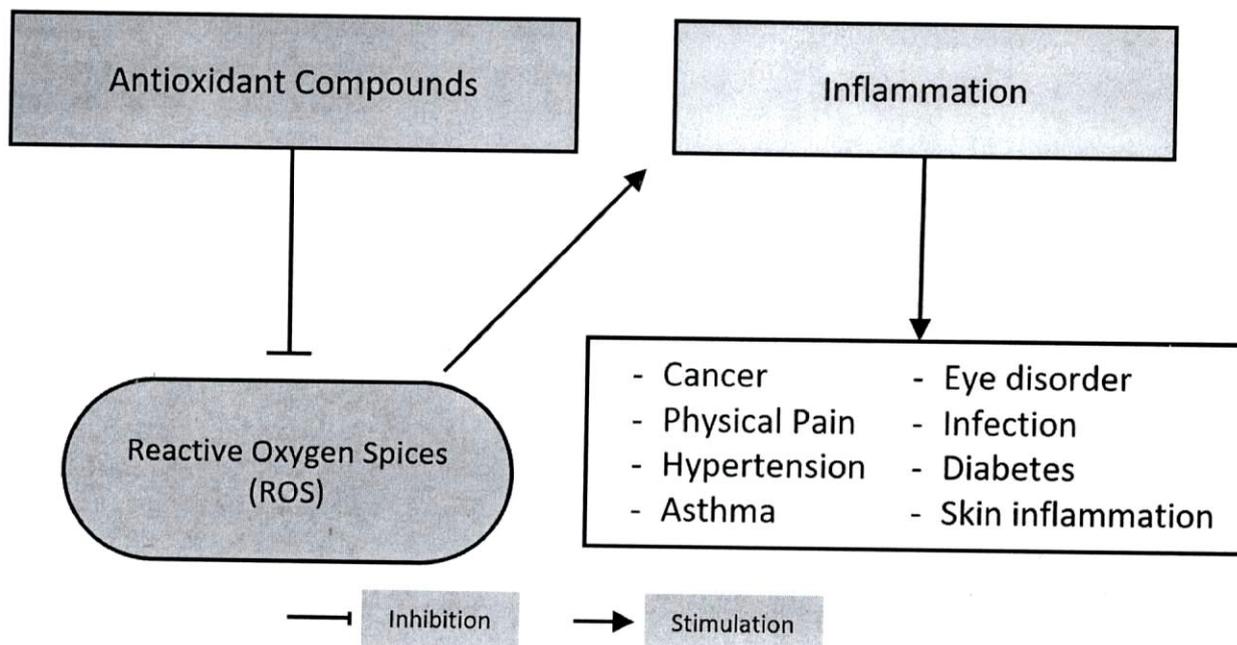
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I. Introduction

Herbal remedies are essential to a thriving community. The lemongrass, or *Cymbopogon flexuosus*, is native to Burma, Thailand, India, and Sri Lanka. Many regions of tropical and subtropical Southeast Asia, as well as Africa, have seen its naturalization. It is one of the plants whose essential oil is said to have excellent potency. The essential oil that is extracted from lemongrass is mostly found in the leaves of the plant (Oniha et al., 2023). It is extensively utilized in the food, feed, cosmetics, and pharmaceutical industries. Strong bioactive components found in lemongrass include terpenoids, alkaloids, and phenolic metabolites (such as phenolic acids, flavonoids, stilbenes, and lignans) (Smija et al., n.d.). Its essential oil is report to have antimicrobial, anticancer, anti-inflammatory and analgesic effect, anti-ulcer and anti-depressant activity. *Melaleuca cajuputi* Powell (MC) is aromatic medicinal plant of the Myrtaceae family that is widely distributed in Asia and Southern Australia. The Major bioactive compounds reported in *M. cajuputi* powell include quinones, flavonoids, phenols, alkaloids, glycosides, 18-cineol, α -pinene, linalool, β -caryophyllene, nerolidol, and terpenoids (Isah et al., 2022). MC is frequently used to cure a broad range of conditions, including thrush, acne, coughing, stomach pains, and bug bites which reported to have a great antioxidant activity against free radical (Arif Azimi Md Noor, 2023).

II. Conceptual framework



(So et al., 2023)

III. Materials and Methods

I. Materials

Analytical grade methanol was purchased from Merck KGaA (Darmstadt, Germany). Trolox was obtained from Thermo Scientific (Fair Lawn, NJ, USA). Quercetin was obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Furthermore, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) was purchased from Thermo Scientific (Fair Lawn, NJ, USA); 1,1-Diphenyl-2-picrylhydrazyl Free Radical (DPPH) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The HPLC standard atropine was purchased from HPC Standards GmbH (Cunnersdorf, Germany). Folin-Ciocalteu's phenol reagent, aluminum chloride, Iron (III) chloride hexahydrate, and hydrochloric acid were purchased from



Merck KGaA (Darmstadt, Germany). The other reagents were purchased from standard commercial suppliers.

II. Plant extraction

In this study, samples used in this investigation were cleaned, dried, and then chopped into tiny bits in an oven (Biobase, BJPX-AUTUMN, China) set at 30 ° C for three hours to remove the water which had attached to the surface area of the plant. 20 g of dried plant was extract with 1% concentrated hydrochloric acid in methanol. The solvent was evaporated by the rotary evaporator (IKA RV10, Selangor, Malaysia) for 30 minutes to preserve the dried residue. The crude extract was stored in the refrigerator for further analysis (So et al., 2023).

III. Phytochemical contents

1. Total phenolic content (TPC) by Folin reagent

The TPC was determined using the Folin-Ciocalteu method (Ghosh et al., 2020). At a final concentration 10 mg/mL of crude extracts were dissolved in methanol. Briefly, 15 µL of extract and 120 µL of prepared Folin-Ciocalteu's reagents were combined, and the mixture was left to sit at room temperature away from light for five minutes. The mixture was then given an additional 90 minutes under the identical conditions after 120 µL of sodium carbonate buffer (pH 7.5) was added. Using the Thermo Scientific™ Multiskan™ FC microplate spectrophotometer, the absorbance of the blue molybdenum (V) solution in Folin-Ciocalteu's reagents was measured at 725 nm. The gallic acid solution was used as a positive control. It was dissolved in methanol (10 mg/mL) and prepared at different final concentrations (5-15 µg/mL) within five experimental replicates. TPC was calculated from a standard gallic acid curve ($y = 0.0589x - 0.0706$, $R^2 = 0.9993$). The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of crude extract.

2. Total flavonoids content (TFC)

The TFC was determined per previous studies (Ghosh et al., 2020). The crude extracts were prepared by dissolving 100 mg of the extract in 1000 µL of methanol. Briefly, 100 µL of the extract was mixed with 50 µL of 2% aluminum chloride as a buffer. Quercetin was dissolved in methanol (10 mg/mL), then diluted at various concentrations (20-60 µg/mL). The experiments were carried out with five replications. The absorbance of the test solution was measured at 415 nm with a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). The TFC was calculated from a standard curve of quercetin ($y = 0.0188x - 0.0522$, $R^2 = 0.9991$). The results expressed the total flavonoid content as milligrams of quercetin equivalent (QE)/g of the crude extracts.

3. Total Alkaloids content (TAIC) via bromocresol indicator

The TALC was performed according to previous studies (Ghosh et al., 2020). The solution of crude extracts was prepared by dissolving 10 mg in 1000 µL of 2N HCL. Briefly, 1000 µL of the extract was mixed with 5 mL of bromocresol 0.2 mM and 5 mL of the citrate phosphate buffer (pH 4.7). The solution mixture was added by 5 mL of chloroform and shaken vigorously. The solution mixture was incubated at room temperature for 30 min. The chloroform layer appeared below the layer of the sample/standard solution, which was collected for analysis at 420 nm using the UV-spectrometry (GENESYS™ 10S UV-Visible Spectrophotometer, Thermo Fisher Scientific, Madison, WI, USA). Atropine, a positive control, was dissolved in 2N HCL at different final concentrations (0.01–0.1 mg/mL). The experiments were carried out in five replications. The standard curve of atropine was created from the plot between the absorbance and the blank solutions, which do not contain any sample mixtures. The TALC was calculated from a standard curve of atropine ($y = 5.9942x + 0.0479$, $R^2 = 0.9997$). The results are expressed as milligrams of atropine equivalent (AE) per gram of crude extracts.



IV. Antioxidant activities

1. DPPH radical scavenging (DPPH)

The DPPH assay was based on hydrogen atom transfer (HAT) reactions. In addition, 2,2-Diphenyl-1-picrylhydrazyl or DPPH will generate a stable free radical with an unpaired electron, delocalized throughout the molecule, producing stabilized molecules (Constantinou et al., 2022). Briefly, various concentrations of the extracts dissolved in methanol (between 100 and 1000 g/mL) and the DPPH reagents were added and mixed in a 1:1 ratio in the 96-well plates for 30 min in the dark at room temperature. The loss of absorbance of the DPPH radical at 515 nm was measured using a microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA). Trolox, a standard antioxidant, was used as a positive control. The linear curve ($y = 0.0052x + 0.015$, $R^2 = 0.9974$) was obtained from the plot between the Trolox concentration and the DPPH radical scavenging power. The experiments were carried out in four replicates. The DPPH radical scavenging capacity was represented as the percentage of DPPH radical inhibition at 50% (IC_{50}). The percentage of inhibition or % of the scavenging effect of DPPH was calculated following Equation (3):

$$\%DPPH \text{ scavenging effect} = [\text{Abs of control} - \text{Abs of sample}] / [\text{Abs of control}] \times 100 \quad (3)$$

- Abs of control = absorbance of control or a reaction mixture in the absence of antioxidant of sample.
- Abs of sample = absorbance of the reaction mixture in the presence of sample.

2. Ferric reduce antioxidant power (FRAP)

The FRAP assay describes the ferric-reducing antioxidant power. This assay method measures the reduction in the ferric ion Fe^{3+} -TPTZ complex to Fe^{2+} -TPTZ (Chunthong-Orn et al., 2016). Various concentrations of each extract, ferrous sulfate, and positive control such as quercetin were dissolved with methanol. The FRAP reagent comprised 300 mM of acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyls-triazine (TPTZ) solution, and 20 mM of $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio. The reaction mixture was pipetted into each well of a 96-well plate and incubated for 30 min in the dark at room temperature. The absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 593 nm with the microplate spectrophotometer (Thermo Scientific™ Multiskan™ FC, Boston, MA, USA) against a blank with methanol. The experiments were tested in five replicates. The standard curve of ferrous sulfate with various concentrations (10-200 μmol) was linear ($y = 0.0042x - 0.0143$, $R^2 = 0.9986$). The FRAP value was calculated from the standard curve and expressed in $\text{mmol } Fe^{2+}$ per gram of extract ($\mu\text{mol } Fe^{2+}/\text{g extract}$).

IV. Results

I. Extraction yield

Two aromatic plants were collected to perform the extraction. *Cymbopogon flexuosus* (CF) and *Melaleuca cajuputi powell* (MC) have been cultivated to extract the methods which have shown the extraction yield below (table 1). The extraction yield has been represented from the highest to lowest is MC (7.69) > CF (4.55).

II. Phytochemical contents

1. Total phenolic content (TPC)

TPC of each plant extraction were equivalent to gallic acid per gram of crude extract (table 2). TPC is represented from the highest to the lowest is MC (9.63 ± 1.26 mg GAE/g of CE) > CF (5.08 ± 1.00 mg GAE/g of CE), respectively.

2. Total flavonoid content (TFC)



TFC of each plant extraction were equivalent to quercetin per gram of crude extract (table 2). TPC is represented from the highest to the lowest is MC (3.32 ± 0.22 mg QE/g of CE) > CF (2.31 ± 0.14 mg QE/g of CE), respectively.

3. Total alkaloid content (TAIC)

TAIC of each plant extraction was equivalent to atropine per gram of crude extract (table 2). TPC is represented from the highest to the lowest is MC (0.12 ± 0.00 mg AE/g of CE) > CF (0.04 ± 0.00 mg AE/g of CE), respectively.

III. Antioxidant activities

1. DPPH

The results are expressed as the percentage inhibition from low to high concentration of sample from 100-1000 $\mu\text{g/mL}$. The chart display as a concentration dependance manner (figure.1). DPPH radical scavenging activities represented the highest concentration of MC (88.39 ± 1.68 %) > CF (55.45 ± 0.28 %), respectively.

2. FRAP

The results are expressed as the micromoles of FeSO_4 per gram of crude extract (figure.1). The results showed the highest ferric-reducing antioxidant power of MC (129.08 ± 6.46 $\mu\text{mol Fe}^{2+}/\text{g}$ extract) > CF (43.79 ± 2.96 $\mu\text{mol Fe}^{2+}/\text{g}$ extract), respectively.

Table 1. A percentage yield of plant extraction.

Plant extraction	CF	MC
Fresh plant weight (g)	24.59	27.57
Percentage Yeild %	4.55	7.69

Table 2. A composition of total phenolic, total flavonoids and total alkaloids contents. Data are expressed as mean \pm SD from five replication.

Bioactive compounds	TPC mg GAE/g	TFC mg QE/g	TAIC mg AE/g
CF	5.08 ± 1.00	2.31 ± 0.14	0.04 ± 0.00
MC	9.63 ± 1.26	3.32 ± 0.22	0.12 ± 0.00

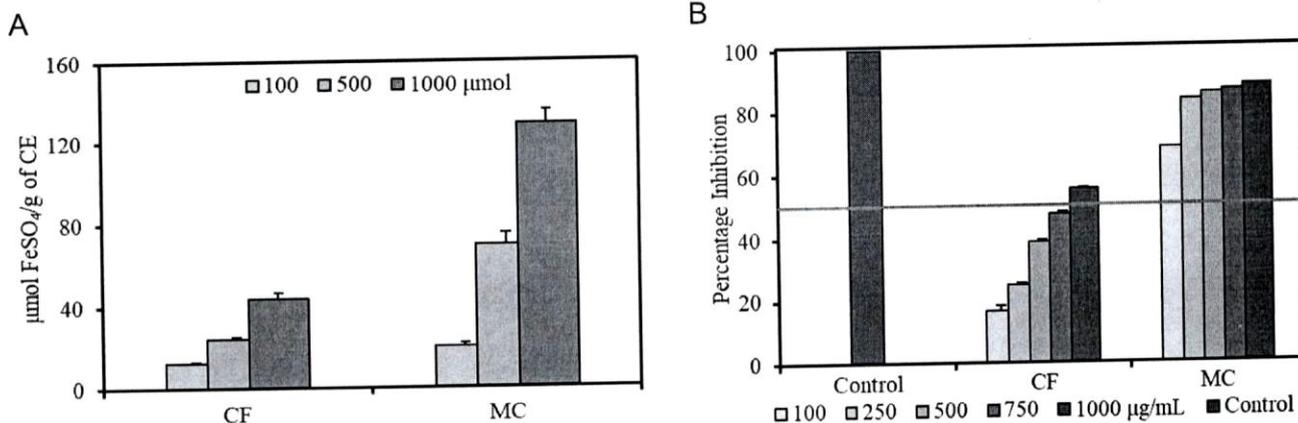


Figure 1. A). FRAP B). DPPH. Antioxidant activities via DPPH radical scavenging activity and FRAP assays. Data are expressed as mean \pm SD from five replicates.



V. Discussion

Comparison of the kasekor product to other products about the compounds such as TPC, TFC and TAIC. The activities of antioxidants such as DPPH and FRAP of each plant. By the report of (Caballero-Gallardo et al., 2022) showed that the total phenolic content in CF 10.6 ± 0.1 mg GAE/g of crude extract and our plants containing 5.08 ± 1.00 mg GAE/g of crude extract. Based on the evidence, CF also represented the antioxidant activities via the cell viability within the cell line HepG2 and Calu-1. It showed the positive way in our experimental that have the power in antioxidant via HAT and SET mechanism by the antioxidant test DPPH & FRAP. The reported of (Al-Abd et al., 2015) indicated that MC flower extract has 55 ± 0.03 and leaves extract 37 ± 0.02 GAE/mg dw and the total flavonoids content that MC flower extract has 19.6 ± 0.4 and leaves extracts 10.2 ± 0.2 QE/mg dw. Based on our results displayed as the aerial part of plant extract containing the total phenolic content 9.63 ± 1.26 mg GAE/g of CE and the total flavonoids content 3.32 ± 0.22 QE/g of CE. Since, the results are equivalent to difference value, so we cannot compare the amount of total phenolic content.

VI. Conclusion

In this study, we found that MC and CF consist large number of phytochemical compounds within high of activities in antioxidant. Within this prove, we can suggest that those plant is qualified in anti-painkiller formulation or production to prevent from the damage of cell which cause by the oxidative stress or free radical.

VII. Reference

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