ANTIOXIDATIVE CONSTITUENTS FROM PETROLEUM ETHER EXTRACT OF *CURCUMA LONGA* LEAVES

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Abstract

Curcuma longa which is traditionally known as turmeric is a well-known plant species with valued medicinal purposes as well as an ingredient in food industry. *Curcuma longa* leaves were believed to contain secondary metabolites with amazing antioxidant properties. Thin layer chromatographic (TLC) technique was implemented to screen antioxidative phytochemicals and preparative thin layer chromatography for isolation purposes. The identification of antioxidative constituents was performed using Gas Chromatography Mass Spectrometer (GCMS) with Wiley database matching individually. TLC screening which revealed petroleum ether extract has higher contribution of antioxidative constituents compared to dichloromethane and methanol extracts and hence it was selected for isolation process of antioxidative constituents. GCMS analysis proposed antioxidative constituents from *C.longa* leaves as **(A)** (2,4cyclopentadien-1-ylidenemethyl) benzene, **(B)**1,3-dimethyl benzene,**(C)**Estra-1,3,5(10)-trien-17β-ol,**(D)**1-methylene-1HIndene,**(E)**1methylcyclopentanol after analyzing with GCMS aided with Wiley Database matching individually. According to its potent antioxidant properties, the use of *C. longa* leaves should be taking into consideration in the development of new natural medicine for the treatment of various diseases.

Keyword: *Curcuma longa*, secondary metabolites, antioxidant activity.

Introduction

Curcuma longa L. commonly known as 'turmeric' is the genus of 70 species of rhizomateous herbs which it is a member of the ginger family (Zingiberaceae). This plant has dark green and large oblong leaves. *C. longa* leaves can be used as flavor to various dishes as they are beneficial for health (Roth *et al.,* 1998). In medicine C. *longa* is used for the treatment of hepatic disorder and rheumatism (Miquel *et al.,* 2002). Secondary metabolites are defined as compounds that derived from primary metabolites that were produced by plants as a defence chemical and some of them can be classified as an antioxidant. Antioxidant is a chemical compound or substance that inhibits oxidation whereby it is a chemical process that leads to the generation of free radicals. The presence of free radicals promote chain reaction to damage cell organism. With antioxidant, this chain reaction will be terminated and finally protects cell from damaging. The most important compounds responsible for the antioxidant activity of *C. longa* were reported as phenolic compounds, such as curcuminoid dyes and essential oils (Antunnes et al., 2012). However, the investigations of antioxidative constituents from non-polar extract of C.longa leaves were rarely found. Based on this circumstance, we aimed to isolate and identify the antioxidative constituents from petroleum ether extract of *C. longa* leaves.

Materials and Methods

Extraction

The fresh leaves of *C. longa* were washed and cut into small pieces and were air-dried at room temperature (26 °C) for 2 weeks. The dried leaves were grinded to a uniform powder. Then 200 g of coarse powder of the fresh leaves of *C.longa* were soaked in 500 ml of petroleum ether, dichloromethane and methanol solvents consecutively with occasional shaking. Each extract mixtures were filtered and evaporated using rotary evaporator. The crude extracts were kept in refrigerator prior to use.

Thin Layer Chromatography (TLC) of Plant Extracts

Each extract was subjected to thin layer chromatography by means to see what and how efficient the components in extract separated on TLC. The extract was spotted on TLC and it was developed in certain binary solvents. The components separated were visualized using Ultra violet light. The development of TLC was repeated until the best separation was obtained on TLC.

Fourier Transform Infra Red (FTIR) Analysis of Crude Extract

The FTIR model of Perkin Elmer Spectrum 100 FT-IR. ATR- FTIR was used to identify functional groups in *C. longa* leaves . The expected functional groups were alkane, alkene, alcohol, carboxylic acid, ester, benzene aromatic ring, amine and carbonyl group.

Qualitative Antioxidant Analysis Using TLC

The TLC plate (1.5 cm x 6 cm) of each crude extract was developed in a suitable developing solvent. After drying process, the TLC plate was sprayed with antioxidant spraying reagent of 0.2 % solution of diphenyl picrylhazine (DPPH) in methanol. The yellow-white spots against purple background indicated the presence of antioxidant constituents. The TLC plate of extracts which demonstrated greater intensity of yellow white spot on TLC plate was considered for isolation of antioxidative constituents.

Isolation of Antioxidative constituents using preparative TLC

The TLC plate of extract which demonstrated greater intensity of yellow white spot was redeveloped using 10 cm x 10 cm TLC plate. After developing process and drying process were completed, the desired band was fully scraped off and was dissolved in its dissolving solvent prior to filtration. A simple filtration process was conducted using cotton wool that was packed into a tiny glass dropper to remove silica gel. The filtrate was left for several hours for drying process at room temperature. The dried compound was kept in refrigerator prior to use.

Gas Chromatography (GC-MS)

The structure identification of antioxidative constituents was performed using Gas Chromatography Mass Spectrometer (GCMS) with Wiley database, in which the isolated compounds were matched with the structure database provided in GCMS individually. The isolated compounds were introduced by using splitless injection of 2.0 µL in methanol fitted with cross linked of 5 % phenyl ethyl siloxane capillary column. Mass detector has been used with the injector temperature was at 220 ºC, with an oven temperature of 60 – 250 ºC with rate of 5 ºC min^{-1}. Carrier gas that has been used was a helium gas at a flow rate of 1.5 mL min^{-1}.

Results and Discussion

Determination of functional groups from FTIR analysis

Table 1 depicted various kinds of functional groups that were found in *C. longa* leaves after they were analyzed with FTIR spectrometer. Conceptually, organic molecules absorbed energy from infra-red radiation when the frequency of infra-red radiation matched with the frequency vibration of covalent bond in molecules and as a result molecules were vibrated at higher amplitude. Since different functional groups of organic molecules absorbed energy of infra-red radiation at different frequency, any functional groups that were presented in any organic molecules can be easily determined by referring to its frequency of vibration or wavenumber (cm^{-1}). Table 1 revealed hydroxyl group, OH stretching at frequency vibration of 3671.60 cm^{-1} which indicated the normal value of hydroxyl group. The absorption frequency of vibration at 2973.53 cm^{-1} corresponded with the C-H stretching, 1822.25 cm^{-1} was for C=O stretching and normally can be recognized as a sharp peak in infra-red spectrum. The presence of C=C aromatic ring in the molecules was recognized from the absorption peak at 1697.04-1486.47 cm^{-1}. The frequency absorption at 1191.68 cm^{-1} belonged to C-O stretching. Therefore, it was suggested that these functional groups might exist in any structural formula of isolated compounds from *C. longa* leaves.

Table 1 Functional groups of *C.longa* leaves

Wavenumber (cm^{-1})	Functional group
3671.60	O-H stretch alcohols, phenol
2973.53	C-H stretch alkanes
1822.25	C=O stretch
1697.04	C=C stretch
1486.47	Aromatic C=C
1191.68	C-O
839.14	C-H bending

Screening of antioxidative constituents from *C.longa* crude extracts

The presence of antioxidative constituents in any plant extract can be easily determined by spraying the developed TLC with DPPH spraying reagent solution. The formation of yellow spot against purple background indicated antioxidative behaviour. The duration of the formation of yellow spot in each extract was closely related to the content of antioxidants in each extract. Figure 1 illustrated TLC of each extract after spraying with DPPH reagent.

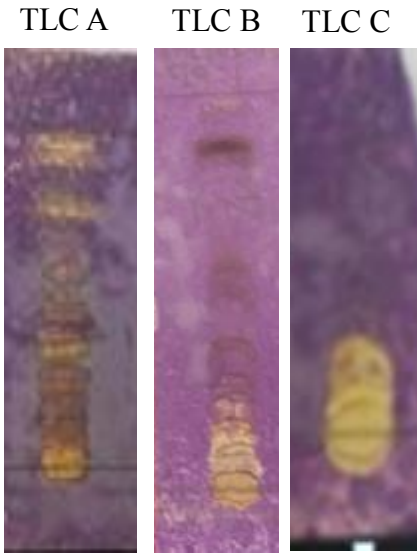


Figure 1 The emergence of yellow spot against purple background in *C. longa* leaves.
TLC A: TLC of petroleum ether extract after spraying with DPPH
TLC B: TLC of dichloromethane extract after spraying with DPPH
TLC C: TLC of methanol extract after spraying with DPPH

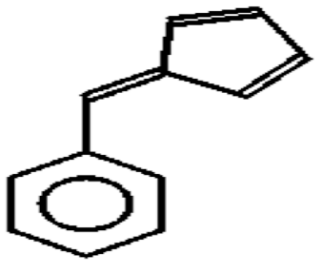
As can be seen in **Figure 1**, the petroleum ether extract seemed to demonstrate higher antioxidants as depicted in TLC A compared to dichloromethane extract and methanol extract. Theoretically, antioxidants donate electrons to free radicals, neutralize and prevent them to cause harm. In our case the DPPH which acts as free radical supplier was neutralized and reduced by the donation of electron from antioxidants in extracts and immediately reduces its colour from purple to yellow as it appeared on TLC. Since TLC A contains greater number of antioxidant, it was chosen for isolation of antioxidative compounds using preparative TLC method.

Proposed antioxidative constituents from petroleum extract of *C. longa*

Five antioxidative compounds were successfully isolated through preparative TLC, whereby compound A and compound B were isolated at retention factor of 0.93 and compound C, D and E were isolated at retention factor of 0.82. All isolated compounds were subjected to GCMS by means of structure determination. Figure 2 illustrates the proposed structures of all isolated compounds after analyzing with GCMS and matching the structures with Wiley Database provided by GCMS.

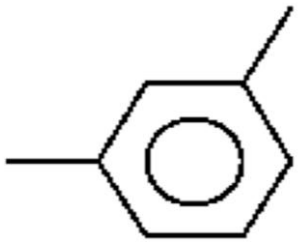
The benzene aromatic systems were found in all compounds except in structure E and the structures were consistent with FTIR data stated about absorption peak of aromatic ring at around 1486 cm^{-1}. Apart from containing benzene ring, compound A and D were also containing normal C=C alkene in which their absorption peaks can also be observed in FTIR data. Compound D and E were the only compounds that carried hydroxyl groups whereby their existence was proved according to the broad absorption peak at area 3671.60 cm^{-1} in FTIR spectrum. Previous investigation reported that curcumin which is one of polyphenolic compound from the rhizomes of *C.longa* was a potent antioxidative compound (Ashraf and Sultan, 2017). They found that the hydroxyl group, OH in curcumin structure may be responsible for the scavenging process of free radicals. Since structure D and E were also carried OH groups, the antioxidant properties of *C.longa* leaves might exist due to the same reason as curcumin.

A



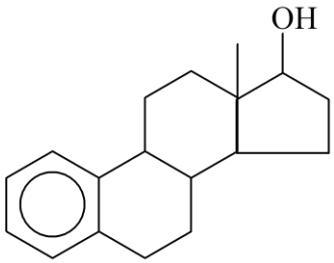
(2,4cyclopentadien-1-ylidenemethyl)benzene

B



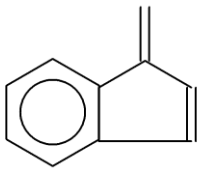
1,3-dimethyl benzene

C



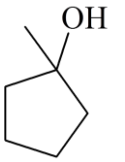
Estra-1,3,5(10)-trien-17β-ol

D



1-methylene-1H-Indene

E



1-methylcyclopentanol

Figure 2 Antioxidative constituents from petroleum extract of *C.longa* leaves

Conclusion

All extracts from *C.longa* leaves have shown its potent antioxidative behavior but petroleum ether extracts is the most. Isolation and identification from preparative TLC and GCMS have proposed 5 antioxidative constituents which namely as **(A)** (2,4cyclopentadien-1-ylidenemethyl)benzene, **(B)**1,3-dimethyl benzene, **(C)**Estra-1,3,5(10)-trien-17β-ol, **(D)** 1-methylene-1H-Indene, **(E)** Cyclopentanol from petroleum ether extract of *C.longa.*

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Conflict of interests

Author declares no conflict of interest.

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