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PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF "UM-MA-RUEK-KA-VA-TEE" HERBAL FORMULA

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Abstract: This research aims to study the phytochemical screening and antioxidant activity of "UM-MA-RUEK-KA-VA-TEE" (UM) herbal formula used as antitussive and expectorant. . The ingredients in UM including myrobalans have been reported for their high amounts of total phenolic compounds that might exhibit a potential benefit as a natural antioxidant. Phytochemical screening of UM was carried out using standard methods of precipitation and coloration reactions. The samples used in this study are 8 products from different manufacturer sources as well as the individual herb extracts. Analysis of antioxidant activity by 1,1-Diphenyl picryl hydrazyl (DPPH) radical assay was reported as IC_{50}. According to the result, *Phyllanthus emblica* extract showed the best antioxidant activity (254.76±0.08 µg/ml). Analysis of antioxidant activity by ferric reducing antioxidant potential (FRAP) assay calculated the ability of FRAP value expressed as mg of Trolox equivalent (TE) per gram crude extract. Samples 3, 7 and 5 had the best antioxidant activity; 242±0.14, 237±0.32 and 216±0.08 mg TE /g crude extract, respectively. Total phenolic content was determined using the standard curve of gallic acid (100-1000 µg/ml). It was found that sample 6 had the highest phenolic content (166 ± 0.06 mg of GA/g of crude extract). Basic phytochemical tests found that 6 samples contained anthraquinones, triterpenoids and tannins. The results from this study will provide the guidelines for the further research and development of UM herbal formula in the future.

Keywords: phytochemical screening, antioxidant activity, herbal formula, Thai traditional medicine.

INTRODUCTION

Thai herbal formula consisted of multiple medicinal plants have been considered as significant therapeutic aid for alleviating ailments of human being and these plants have been used for treatment of various ailments since ancient time. Besides the rapid development of methods of chemical synthesis in laboratory, medicinal plants continue to play an important role as the alternative treatment to modern medicine due to the combination of their distinct biological activities (Ngo *et al*., 2013).

"Um-Ma-Ruek-Ka-Va-Tee " (UM) is a traditional Thai polyherbal formula included in Thailand national list of essential medicine(NLEM) as antitussive and expectorant. The major

ingredient in the formula is licorice root (50 %), the equal part of five herbs including chebulic myrobalan (*Terminalia chebula* Retz.), beleric myrobalan(*Terminalia belerica* Roxb.), emblic myrobalan or Indian gooseberry (*Phyllanthus emblica* L), white cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.) and Terminalia galls (galls from the infected leaves and young shoots of *Terminalia chebula* Retz.); each of them is counted for 10 % of the formula. Licorice root is derived from three plant species, *Glycyrrhiza* uralensis, *G. glabra*, and *G. inflata*.UM formula from Thailand NLEM is the modified formula that removed one medicinal plant from the original formula due to the presence of aristolochic acid (Debelle *et al*., 2008). UM products are available in two dosages forms including powder and honey boluses prepared by mixing fine powder of all ingredients with honey.

Three ingredients from UM formula, chebulic myrobalan, beleric myrobalan and Indian gooseberry are also known as the 'three myrobalans' or Triphala. Triphala is a frequently used herbal medicine in India and Thailand to treat many diseases such as jaundice, constipation, asthma, fever and chronic ulcers (Deepa, 2013; Jagetia *et al*., 2002). There are plenty of antioxidant studies from Triphala formula which reveal the powerful antioxidant action (Parveen *et al*., 2018). Other than the three myrobalans, UM formula contained 50 % of licorice, the rich source of essential oils from white cumin and coriander, main source of tannins from Terminalia galls. Licorice root, the main ingredient of UM, contains the glycoside, glycyrrhizin which has a similar structure and activity as the adrenal steroids. Licorice has an anti-inflammatory activity similar to cortisone and has been found useful for arthritis and allergies (Jatav *et al*., 2011).

According to our extensive literature reviews, there is no previous study on the phytochemical and antioxidant study of UM. Physicochemical screening and antioxidant study of the plant material is the preliminary and key aspect to establish the potential of plants based on their chemical constituents. Due to the unique formulation of UM formula contained astringent medicinal plants (three myrobalan) combined with plants with sweet taste (licorice) and hot spicy taste (coriander and cumin), this formula has a tendency to be preventive medicines form the antioxidant action. 1,1-diphenyl-2-picrylhydrazine (DPPH) assay and Ferric ion reducing antioxidant power (FRAP) assay are selected for the antioxidant study. Both of selected assays are spectrophotometric methods, which are used to investigate the in vitro antioxidant capacity of foods and biological samples. They are based on electron transfer reactions, which visually results on the reduction of a colored oxidant (DPPH or FRAP as oxidant). Therefore, usually the results obtained from these methods present excellent correlation. The major aims of this study are to perform the antioxidant study and phytochemical study of UM formula compared with the individual herb**.** Screening of the antioxidant activity of medicinal plants or herbal formula will provide the data of free radical scavenging activity which in turn provide the understanding the role of particular plants in minimizing the oxidative stress linked pathophysiology of diseases (Aruoma, 1994).

MATERIALS AND METHODS

Sample collection

All the sample of raw materials were identified and authenticated in the Department of pharmacognosy and toxicology, Faculty of pharmaceutical sciences, Khon Kaen University, Thailand.

In this study, 7 commercial samples of "Um-Ma-Ruek-Ka-Va-Tee (UM)" were randomly purchased from the drug store in Thailand. In-house sample of UM was prepared from six ingredients as powders by mixing them in based on formula of Thai traditional medicine (6 parts of licorice and one part for each of the other five ingredients). In house product

was randomly assigned as product No 7. Other commercial products were assigned as product No 1-6 and No.8.

Preparation and Extracts

0.0010 g of eight UM samples were weighed and dissolved with boiled water (40-50 °C) and left for 15-20 minutes until the medicine is completely dissolved. Then each sample was filtered using syringe filter and use for the phytochemical screening and antioxidant study. Each sample were freshly prepared for each experiment. Four major ingredients of UM formula, *Glycyrrhiza glabra* Linn., *Coriandrum sativum* L. *Terminalia belerica* Roxb.and *Phyllanthus emblica* Linn.,were prepared using the same procedure as UM samples. The single herb extracts were used to compare the total phenolic content and antioxidant activity with the UM samples.

DPPH Radical Scavenging Assay (DPPH)

0.2 mM DPPH readical solution and 10,000 ppm of sample was prepared in methanol. The sample was then diluted to get the range of concentration from 10-1,000 ppm. DPPH in methanol was then added to 1 mL of various concentrations of each extract to be tested. After 30 min at room temperature, the absorbance of the reaction mixture was measured at 517 nm compared to the standard Trolox. Ascorbic acid was used as positive control. All the concentrations were performed in triplicates (n = 3) and then calculate for the percentage of radical scavenging and IC_{50} from the equation as follows:

$$\% \text{ radical scavenging} = [1 - (A_{sample} / A_{control})] \times 100.$$

Ferric reducing antioxidant power (FRAP)

The Ferric reducing antioxidant power (FRAP) reagent was prepared by mixing 300 mM acetate buffer, 10 ml 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$.6H2O in the proportion of 10:1:1 at 37°. Freshly prepared working FRAP reagent was pipetted using 1-5 ml variable micropipette (3.995 ml) and mixed with 5 µl of the appropriately diluted sample and mixed thoroughly. An intense blue color complex was formed when ferric tripyridyl triazine (Fe3+ TPTZ) complex was reduced to ferrous (Fe2+) form and the absorbance at 593 nm was recorded against a reagent blank (3.995 ml FRAP reagent+5 µl distilled water) after 30 min incubation at 37°. All the determinations were performed in triplicates (n = 3). The calibration curve was prepared by plotting the absorbance and different concentrations of $FeSO_4$. The concentrations of $FeSO_4$ were in turn plotted against concentration of standard antioxidant Trolox expressed as mg of Trolox equivalent per gram of sample.

Determination of Total Phenolic Content

Total phenolic content (TPC) was determined by Folin-Ciocalteu reagent based spectrophotometric assay with slight modifications. 10 mg of each crude extract was dissolved in 1 mL of corresponding extracting solvent to produce the stock of sample solution at 10,000 ppm. The lower concentrations of sample at 500 and 1000 ppm were prepared by diluting the stock sample solution. 0.5 ml of sample was put in a test tube and mixed with 2.5 ml of freshly prepared 10% Folin-Ciocalteu reagent, followed by an addition of 2.0 ml of 7.5% sodium carbonate after 5 minutes. Then, the resulting mixture was incubated at 40°C for an hour and absorbance was measured at 765 nm after incubation. Gallic acid was used as calibrated standard and results were expressed as milligram gallic acid equivalent per gram of

dry weight (mg GAE/g dry weight). The content of phenolics for each extract was determined in triplicate. All results were averaged, and data was reported as mean ± SD.

Phytochemical screening

The aqueous and methanolic extracts along with other solvent extracts of raw materials were studied for presence or absence of various phytochemicals like alkaloids, tannins, anthraquinones and cardiac glycosides, saponins, and terpenoids by using precipitation and coloration reactions. Methanol and water extract prepared by 10 g of dried powder taken in 50 mL of solvent (methanol or water) in a conical flask, plugged with cotton wool and then kept on a rotary shaker for 24 hours. After 24 hours, the extract solvents were filtered, the filtrates were collected, evaporated and the extracts were stored at 4°C in airtight bottles. Powders or extract of the each plant materials was then analyzed by specific reactions; the color of the extracts and precipitates were observed for identification. (Sorsavanh, 2019).

Test for Anthraquinones

10 ml of 10% solution (H_2SO_4) was added in 0.2 g of the extract in a conical flask and put in the heated water bath for 10 minutes and then filtered. Filtrate was extracted 2-3 times with the equal portion of chloroform. 10 ml of ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

Test for Terpenoids

: Approximately 2 mg of dry extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated the test as positive for triterpenoids.

Test for Saponins

0.2 g crude extract was shaken vigorously with 5 ml distilled water in a test tube. Formation of froth was taken as positive test for presence of saponins.

Test for Tannins

1 g of powdered sample was separately boiled with 20 ml water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5ml with distilled water and three drops of 10% ferric chloride. A brownish- green precipitate indicated the presence of tannins.

Test for Alkaloids

0.2 g of dry extract was dissolved with 15 ml of 2 % sulfuric acid. Extract was left at room temperature for 2-3 minutes and then filtered. A few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to 2- 3ml of filtrate. Formation of orange red precipitate indicates the presence of alkaloids.

Test for Cardiac Glycoside

5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. The

formation of the brown ring at the interface indicated the deoxy suger characteristics of cardenolides. A violet ring appeared below the ring while in the acetic acid layer, a greenish ring was formed.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Assay (DPPH)

According to the results of DPPH radical scavenging assay of the extracts. The IC_{50} of the extracts were ranged from 84.46 µg/ ml to 846 .07 µg/ ml (Figure 1). The order of DPPH scavenging activity of UM and plant extracts were found to be in the order of *Phyllanthus emblica* extract, No1, No3, No2,No6, *Terminalia bellerica* extract, No8, No7, No 4, *Coriandrum sativum* extract and *Glycyrrhiza glabra* extract. However, the strongest DPPH scavenging activity was the positive control, ascorbic acid, with the IC_{50} value of 84.46 ±0.03 µg/ ml.

Ferric reducing antioxidant power (FRAP)

Overall, the anti-oxidative stress activity of the extracts varied from 34 ± 0.02 to 242 ± 0.14 mg Trolox equivalent/g extract (Figure 2). Among all the extracts, sample No. 3 showed the highest FRAP value (242 ± 0.14 mg TE/g extract), followed by No 7 (237 ± 0.32 mg TE/g extract) and No5 (216 ± 0.08 mg TE/g extract). All UM formula exhibited the higher FRAP values than individual herbs. *Glycyrrhiza glabra* extract has the lowest anti-oxidative stress activity with the FRAP value of 34±0.02 TE/g extract.

Total Phenolic Content

Total phenolic content was calculated from the standard curve of gallic acid with equation $Y = 0.0296x + 0.2519$ ($R^2 = 0.9896$) The results exhibited that all UM formula has the higher total phenolic content than the individual herbs with the content varied from 60 to 166 mg GAE g crude extract (Table 1). The highest total phenolic content was sample No.6 (166±0.06 mg GAE g crude extract) followed by sample No 4. (146±0.03 mg GAE g crude extract). *Glycyrrhiza glabra* extract showed the lowest total phenolic content (27 ±0.03 mg GAE/g crude extract).Among the individual herbs, *Phyllanthus emblica* extract has the highest total phenolic content. According to the results, four single herb extracts exhibited the same ranking for total phenolic content and FRAP assay.

Table 1. Total Phenolic Content from UM samples (mg GAE / g crude extract)

Sample name	Total phenolic content (mg GAE/g crude extract)
No. 1	75±0.05
No. 2	68±0.10
No. 3	72±0.02
No. 4	146±0.03
No. 5	60±0.07
No. 6	166±0.06
No. 7	86±0.07
No. 8	94±0.09
Glycyrrhiza glabra Linn.	27±0.03
Coriandrum sativum L.	36±0.05
Terminalia belerica Roxb.	31±0.04
Phyllanthus emblica Linn.	57±0.02

Determination of Phytochemical screening

Screening results indicated that all the samples of Um-Ma-Ruek-Ka-Va-Tee exhibited the positive results for anthraquinone, terpenoid and tannin test. Majority of UM samples showed the positive results for saponin test except NO. 2 and NO.4 Alkaloids and cardiac glycosides were not detected in all UM samples (Table 2). The positive results on anthraquinones and tannins were related to three crude drugs chebulic myrobalan, beleric myrobalan and emblic myroblan that provide the astringent and laxative action of these fruits. The positive result on tritepenoid test correlated to the major ingredient, licorice root, which is well known for the triterpenoid saponin compound called glycyrrhizin. Glycyrrhizin is a glycosides and also a triterpenoid saponin due to its structure derived from oxidized polymers containing 30 carbon atoms. The positive screening test for both saponin and triterpenoid test were somewhat confirm the presence of glycyrrhizin in UM formula but further HPLC study is required for the exact conclusion.

Table 2. Phytochemical screening results of UM samples

Samples	Anthraquinones	Terpenoids	Saponins	Tannin	Alkaloids	Cardiac Glycoside
No.1	+	+	+	+	-	-
No.2	+	+	-	+	-	-
NO. 3	+	+	+	+	-	-
NO.4	+	+	-	+	-	-
NO. 5	+	+	+	+	-	-
NO. 6	+	+	+	+	-	-
NO. 7	+	+	+	+	-	-
NO. 8	+	+	+	+	-	-

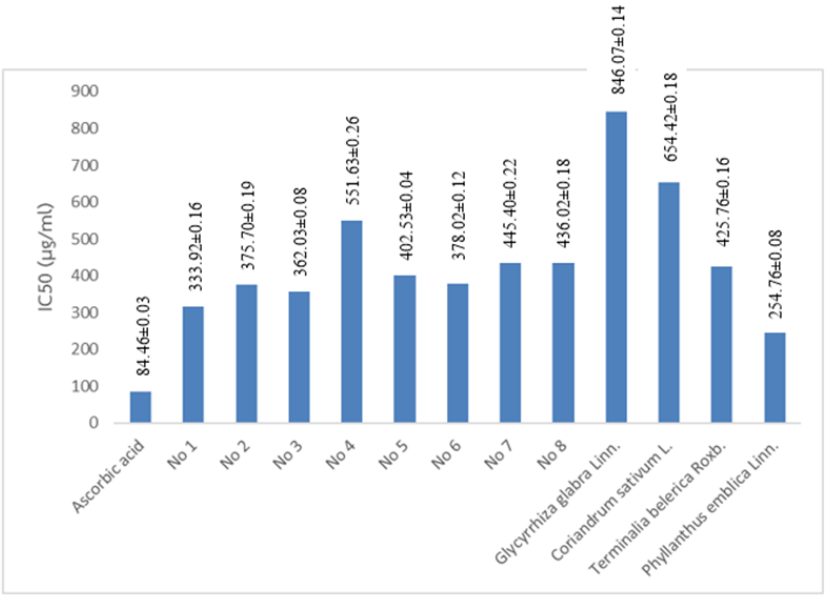

Figure 1. IC_{50} values of DPPH Radical Scavenging of UM. Samples and single herbs

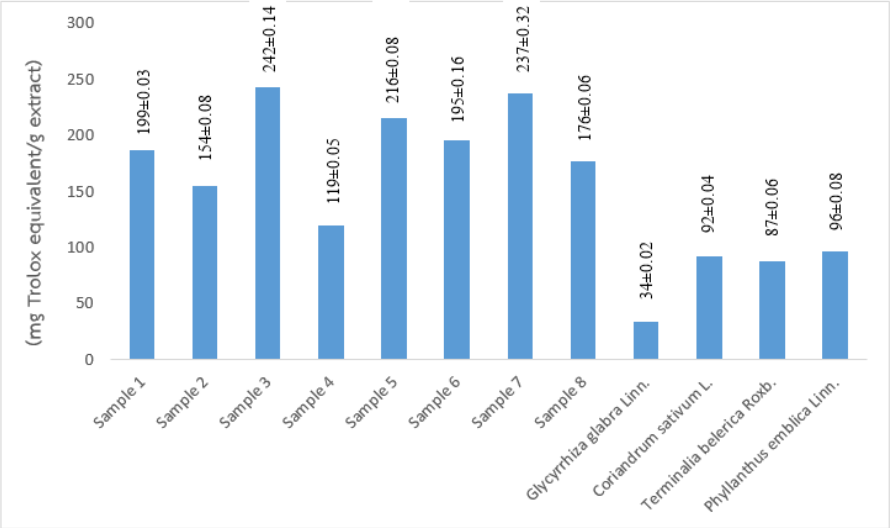

Figure 2. FRAP assay value represent as Trolox equivalence /g of UM crude extract in concentration 1000 µg/ml.

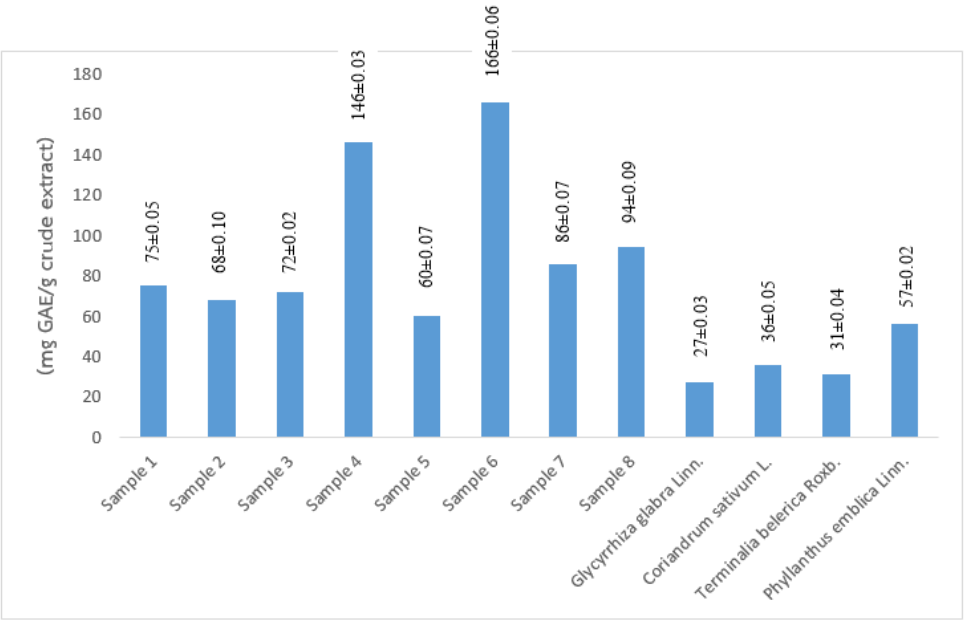

Figure 3. Total Phenolic Content represent as mg GAE/g crude extract

CONCLUSION

The aim of this study was to test whether Um-Ma-Ruek-Ka-Va-Tee used for traditional medicine practices could be promising sources of natural antioxidants. For the first time, the phytochemical constituents of UM formula are identified qualitatively as a preliminary study. The presence of triterpenoids and tannins show the high medicinal value of this plant as antimicrobial and antidiarrhoeal corresponded to the three myrobalan fruits (Triphala) as a part of this formula (Biradar *et al*., 2007). The positive phytochemical tests for anthraquinones and tannins also confirmed the characteristics of Triphala as laxative (Peterson *et al*., 2017). The

positive results on triterpenoids confirmed the chemical characteristics of licorice due to its major constituents, glycyrrhizin (Pastorino *et al*., 2018). The presence of triterpenoids is likely related to glycyrrhizin which is the main ingredient from licorice which is used as 50 % of UM formula.

It was observed that the total phenolic contents of all individual herbs correlates well with DPPH assay and FRAP assay. Majority of UM samples showed an inconsistency pattern of ranking with DPPH assay and FRAP assay. Overall, UM formula exhibited the higher total phenolic content and FRAP value than the individual herbs. However, *Phyllanthus emblica* extract showed the best antioxidant activity with IC_{50} of 254.76±0.08 µg/ml. With the exception of *Phyllanthus.emblica* extract, all UM samples exhibited the stronger antioxidant activity than the individual herbs in DPPH assay. Ascorbic acid was chosen as the positive control for DPPH assay and was the best anti-oxidative stress activity with the IC_{50} of 84.46±0.03 µg/ml. Sample No.3 obtained the highest FRAP activity and the third in term of rankings for DPPH assay. *Glycyrrhiza glaba* extract which is the major ingredient of UM formula exhibited the lowest total phenolic content as well as the lowest antioxidant activities for both DPPH assay and FRAP assay. According to these results, the antioxidant of UM formula may contribute from the combination of all ingredients, especially *Phyllanthus emblica*, other than its major ingredient. It is important to note that all FRAP values of UM samples were much higher than the values of individual herb. The antioxidant capacity determined by FRAP assay suggests the potential of UM formula as the preventive products for antioxidant action besides its indication as antitussive and expectorant.

Further study on the standardization of UM formula which the appropriate chemical markers elucidation of the chemical composition and in vivo studies are necessary in order to better establish the functionality of UM formula as preventive health products.

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