# Formulation of Capsule Dosage Form Containing Ethanolic Fruit Extract of *Terminalia chebula* Retz. (Hpan-ga) Having Potent Antioxidant Activity

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Recently, the usefulness of medicinal plants in health care delivery has been emphasized and WHO recognizes and endorses the use of those which have been scientifically proven to be efficacious, safe for use and of good quality. Thus, it is necessary to formulate these valuable herbal medicinal plants into dosage form. This study is aimed to formulate the capsule dosage form containing 75% ethanolic fruit extract of *Terminalia chebula* Retz. (Hpan-ga) having antioxidant activity. Prior to formulation into capsule dosage form, physicochemical and phytochemical characterization were investigated and all were complied with the specified limits prescribed in the Pharmacopoeias. In addition, according to the Folin-Ciocalteu colorimetric method, the total polyphenol content of 75% ethanolic extract was 270.664 mg GAE/g and $IC_{50}$ value obtained from DPPH radical scavenging test was 0.238 mg/mL. Thus, ethanolic extract having antioxidant activity was formulated into capsule dosage form using four different formulations containing adsorbent-loaded dried extract. The pharmaceutical evaluation studies revealed that among different formulations, microcrystalline cellulose (MCC)-loaded dried extract capsule containing 3% talc showed short disintegration time ($p<0.05$), good uniformity of weight and dissolution. Thus, according to the study, capsule containing MCC-loaded dried extract granules with considerable amount of total polyphenol content showed reproducible capsule dosage form having antioxidant activity.

*Keywords:* Total polyphenol content, Antioxidant, DPPH, Capsule dosage form, MCC, Starch

## INTRODUCTION

In recent years, plant-derived products are increasingly used as medicinal products, nutraceuticals and cosmetics. Less toxicity, better therapeutic effect, good patient compliance and cost effectiveness are the reasons for choosing drug from natural origin. Among the herbal medicinal products, the global interest is the usefulness of antioxidant activity in herbal medicine. Inhibition of free radical-induced oxidative damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases. Among natural antioxidant, phenols and polyphenols are promising phytochemical as important class of natural products having multiple polar functionality and a variety of effects towards antioxidant behavior. In Myanmar, there are many indigenous medicinal plants which claimed traditionally for health benefits. Among these, *Terminalia chebula* Retz. (Combretaceae) is well-known plant in Myanmar and is called Hpan-ga. Because of several medicinal properties attributed to

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*Terminalia chebula* Retz., it is used extensively in the preparation of many Ayurvedic formulations. The antioxidant activity is promising in the fruit of *Terminalia chebula* Retz. because of the major constituent of tannin (32%) especially hydrolysable tannin such as gallic acid, ellagic acid and corilagin which are natural polyphenols possessing strong antioxidant, anticancer, antimicrobial, and anti-inflammatory vactivities.[1] However, according to Fang & Bhandari (2010), these valuable natural compound's uses are substantially limited because of their instability during food processing, distribution or storage.[2] Other problems in relation with uses of polyphenol in human health are unpleasant taste which must be masked before their incorporation in foodstuffs or oral medicines. Therefore, the administration of phenolic compounds requires the formulation of a finished protecting product able to maintain the structural integrity of the polyphenol until the consumption or the administration, mask its taste, increases its water solubility and bioavailability.

For efficacy of the medicinal plant, not only the effectiveness of the plant or extract on the treatment but also the presentation of the dosage form are needed to be considered. Although the most common dosage forms of herbal preparations are liquids derived from macerations, infusions and decoctions,[3] this type of dosage forms have some associated problems of large dose volumes, impractical packaging and poor stability.[4] Solid preparations on the other hand often have higher stability and are easier to standardize which adds to an increase in their therapeutic acceptance, efficacy and product value.[5]

Among oral solid dosage form, capsule dosage form is preferred. This may be due to the fact that capsule dosage form needs not much formulation steps and it may give protection of ingredients from environmental condition. Moreover, these dosage form can mask the bitter taste and unpleasant smell of herbal extract.

The identification of the active compounds in the herbal medicine is important to know how much the active components intake by patient for single doses. Therefore, the standardization of the total polyphenols content in dosage form is important factor.

The present study is aimed to formulate the capsule dosage form containing ethanolic fruit extract of *T. chebula* with high phenolic content and antioxidant activity. The formulation of standardized ethanolic fruit extract of *T. chebula* into a pharmaceutical capsule dosage form would gain the benefiting effect of oral dosage form. Therefore, this finding may benefit in the development of newer herbal drugs from locally available medicinal plant and this may provide a useful standardized herbal dosage form to public.

## MATERIALS AND METHODS

### *Investigation of phytochemical and physicochemical study*

The present study was laboratory-based analytical study. The fresh mature fruits of *Terminalia chebula* Retz. were collected from Magway Division in May and the authenticity was confirmed by competent botanist. The preliminary phytochemical screenings were qualitatively determined according to Harbone (1984) [6] and the physicochemical properties of *T. chebula* Retz. were assessed according to the quality control methods for medicinal plant materials.[7, 8] The analysis was conducted at University of Traditional Medicine, Mandalay.

### *Extraction of fruits of Terminalia chebula* Retz.

According to the previous study, the 75% ethanol was found to be the suitable solvent for extraction of *T. chebula* fruit to obtain high amount of total polyphenol.[9] Dried powder was extracted with 75% ethanol by cold maceration process for 7 days. Then, 75% ethanolic extract was filtered and concentrated at 40ºC under reduced pressure by rotary evaporator. The obtained liquid extract was then dried on water bath at 50°C

for 48 hours until the concentrated viscous extract was obtained.

*Acute oral toxicity test*

The ethanolic extract of *T. chebula* Retz. was tested for acute toxicity according to Organization of Economic Cooperation and Development guideline 425[10] which was performed at Pharmacology Research Division, Department of Medical Research, Yangon. According to the literature, the ethanolic extract of *Terminalia chebula* Retz. is likely to be non-toxic.[11] Therefore, limit test at 5000 mg/kg was performed.

*Determination of total phenol contents in the ethanolic fruit extract of T. chebula* Retz

The major constituent that gives antioxidant activity of *Terminalia chebula* fruit is total polyphenol. This experiment was carried out according to Celep, Aydin & Yesilada (2017) with some modification.[12] Determination of total phenolic compounds was done by Folin Ciocalteu colorimetric method and the absorbance was measured at 765 nm with UV-Vis spectrophotometer. Then, the results were expressed as gallic acid equivalent (GAE) (n=3).

*Determination of in vitro antioxidant activity of ethanolic extract by DPPH radical scavenging assay*

Antioxidant activitiy of ethanolic extract was determined by *in vitro* DPPH method according to Vieira *et al*. (2013) with some modification.[13] The ascorbic acid was used as standard. The sample solutions were prepared by dissolving the extract in 75% ethanol to obtain the concentrations of 10, 40, 80, 120, 160, 200 µg/mL, respectively. To 2.9 mL of DPPH (60µM) solution, 0.1 mL of extracts with different concentrations were added and mixed vigorously by a vortex mixer. All solutions were allowed to stand at room temperature for 30 minutes in the dark and measurement of absorbance was done at 517 nm using a UV-Vis spectrophotometer. The percent inhibition was calculated by using the following formula.

$$\text{Percent (\%) inhibition} = \frac{\text{A control - A test}}{\text{A control}} \times 100$$

Where, % inhibition = the percent inhibition of sample to be tested

A control = The absorbance of 60 µM DPPH solution

A test = The absorbance of test sample solution

The concentration of sample required to scavenge 50% of DPPH free radical ($IC_{50}$) was calculated from the linear regression line of radical scavenging activity (% inhibition) against the concentration of extracts.

*Formulation of capsule dosage form*

In order to formulate into capsule dosage form, dry powder of extract was required. Thus, viscous ethanolic extract was mixed with the adsorbent individually with 1:1 ratio. The two adsorbents used were microcrystalline cellulose (MCC) and starch. Then, the damp mass were placed on the tray and dried in the oven at 50˚C until completely dried. Then, the dried products were sieved through 20 mesh sieves to obtain uniform granules.

Table 1. Formulation of capsules

| Code | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Types of adsorbent added | Starch | Starch | MCC | MCC |
| Adsorbent-extract granules (mg/cap) | 450 | 450 | 450 | 450 |
| Lactose granules (mg/cap) | 45 | 35 | 45 | 35 |
| Talc (1% & 3%) (mg/cap) | 5 | 15 | 5 | 15 |
| Total weight (mg/cap) | 500 | 500 | 500 | 500 |

The preparation of different formulations of capsule dosage form and the pharmaceutical evaluation were performed at University of Pharmacy, Mandalay. The capsules containing nominal weight of 450 mg adsorbent extracts granules were prepared (Table 1). Prior to mixing and filling into capsule, granulation of lactose (5%) was done. Then, dried adsorbent-extracts powder was mixed with the lactose granules whereas the lubricant (talc) with two different concentrations (1% and 3%) were also added to give good flow property of herbal capsule. Then, the obtained granules were filled into

hard gelatin capsule, size No. 0. The hand-operated hard gelatin capsule filling machine was used for filling the powder mixed into the capsule shell and the obtained capsules were cleaned and polished by capsule polishing machine to remove dust and powder on.

*Evaluation of capsule*

Prior to capsule filling, the obtained granules were evaluated for moisture content, bulk density, tapped density, Carr's index and Hausner's ratio. Compatibility test was also carried out. The evaluation of the quality of finished herbal capsule was carried out using pharmacopoeia and non-pharmacopoeia test. Determination of pH, size and shape of the capsule, uniformity of weight, determination of total phenol content in capsule, disintergration and dissolution were carried out. All parameters were carried out triplicate except for disintegration time which was carried out six times.

## RESULTS AND DISCUSSION

Phytochemical test revealed the presence of polyphenols, tannins, saponins, amino acids, glycosides, carbohydrates, steroids and terpenes whereas cyanogenic glycoside, the natural plant toxin, was not detected in *Terminalia chebula* fruit. According to the physicochemical parameters of the fruit of *T. chebula*, total ash value content was 4.11% whereas the amount of acid-insoluble siliceous matter present was 1.025% and water-soluble ash in fruit gave 1.6%. In the present study, the water and volatile matter at 105°C was found to be 6.87%. The extractive values showed 27.4% of ethanolic extract and 43.12% of watery extract and 34.1% of petroleum extract, respectively.

*Determination of the total phenol content and antioxidant activity*

The total polyphenol content and the antioxidant activity were conducted at Pharma- cology Research Division, Department of Medical Research, Yangon. The amount of total polyphenol was found to be 270.664 mg GAE/g for viscous extract. In determination of DPPH radical scavenging assay, the ratio of extract and DPPH radical (60µM) used in this study was 0.1: 2.9. The smaller $IC_{50}$ value corresponds to a higher antioxidant activity of the plant extract.[14]

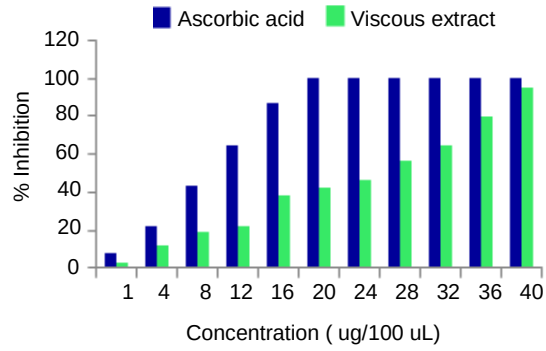


Fig. 1. Comparison of DPPH radical scavenging activity

The radical scavenging activity of viscous ethanolic extract was the highest at the maximum dose of 40 µg/100 µL while that of standard ascorbic was at 20 µg/100 µL (Fig. 1). The scavenging activity of extract gradually increased as the concentration increased which proved the concentration dependent DPPH radical scavenging activity. *T. chebula* fruit extract has antioxidant activity with an $IC_{50}$ values of 0.238 mg/mL for concentrated viscous extract whereas for ascorbic acid, $IC_{50}$ was 0.093 mg/mL which means the antioxidant activity of standard ascorbic acid was greater than that of the extract in three times owing to the pure active properties of ascorbic acid and the pure compound possess high radical scavenging activity. Thus, the total polyphenol should be isolated from extract and formulated into dosage form so that it can give comparative $IC_{50}$ value like standard ascorbic acid. One study showed that $IC_{50}$ for ascorbic acid was 0.127 mg/mL which gave 3.6-fold higher activity than that of the aqueous extract of *T. chebula* from Korea.[15]

*Acute toxicity test*

In this experiment, no lethality of the mice was observed up to 14 days, even with dose of 5000 mg/kg body weight of 75% ethanolic extracts. Thus, the $LD_{50}$ cut-off value was expected to be greater than 5000 mg/kg.

*Evaluation of capsule*

Prior to evaluation of finished capsules, the compatibility study between extract and excipients was studied and the result revealed that the FTIR spectra of extract exhibited the major peak of phenolic group whereas the binary mixtures exhibited all the main absorption bands of extract. Hence, it can be concluded that the spectrum of binary mixtures approved no significant changes in major peaks of extract (data not shown). Lower angle of Repose, Carr's index and Hausner' ratios of a material indicated better flow properties than higher ones and MCC-loaded extract granules gave lower Carr's index and Hausner's ratio than starch-loaded extract granules (Table 2). Good flow properties of granules enhanced uniform filling of capsules and based on the results obtained, flow of property of MCC-loaded extract granules indicated the more acceptable one.

Table 2. Determination of granules flow properties and evaluation of uniformity of weight and disintegration

| Codes | Moisture content | Carr's Index (%) | Hausner's ratio | Angle of repose (Degree) | Size in diameter (mm) | Uniformity of weight (g) | Disintegration time (min) |
|---|---|---|---|---|---|---|---|
| F1 | 4.4% | 40.00 | 1.7 | 29.7 | 7.30 | 0.486 | 8.17 |
| F2 | 4.6% | 41.3 | 1.7 | 35.1 | 7.25 | 0.489 | 8.1 |
| F3 | 4.7% | 39.5 | 1.7 | 21.8 | 7.25 | 0.489 | 3* |
| F4 | 4.4% | 39.7 | 1.7 | 24.8 | 7.25 | 0.493 | 3.66* |

The esteric (*) represents the statistical significance at 0.05 level.

The pH for 1% solution of F1, F2, F3 and F4 were 4.48, 4.48, 4.25 and 4.23, respectively. The uniform length of the capsules was in the range of 21.5 to 21.6 which proved the cap of the capsule tightly fit with body of the capsule. The uniformity of weight test of formulated capsules may indicate whether the granules used for encapsulation possessed good or poor flow properties. Good flow properties of granules will enhance the uniform filling of capsules in capsule filling machines.[16] Regarding to the flow property of granules, MCC-loaded extract capsules gave better flow than the other formulated capsules and this observation was corresponded with uniformity of weight.

The total polyphenol content for F1, F2, F3 and F4 were 72.9, 80.6, 80.2 and 83.5 mg GAE/cap, respectively, whereas capsule containing MCC-loaded extract granules (F4 with high percentage of talc) gave higher amount of polyphenol than that of capsule. Owing to the better drying efficacy of MCC, the small amount of MCC can adsorb large amount of extract. Disintegration is a very important step for the drug bioavailability and absorption. In this study, the disintegration time for capsules containing MCC-loaded extract granules (F3 and F4) disintegrated more rapidly than starch-loaded extract capsules (F1 and F2) ($p<0.05$). This finding was corresponded with Uwaezuoke *et al*.[17] And it also revealed that the adsorbents used in the present study such as MCC and starch function not only as an adsorbent but also as disintegrant. In the present study, the action of the MCC as disintegrant may be proposed by providing the capillarity to draw in liquid into compacts for separation of bonded particles.[18]

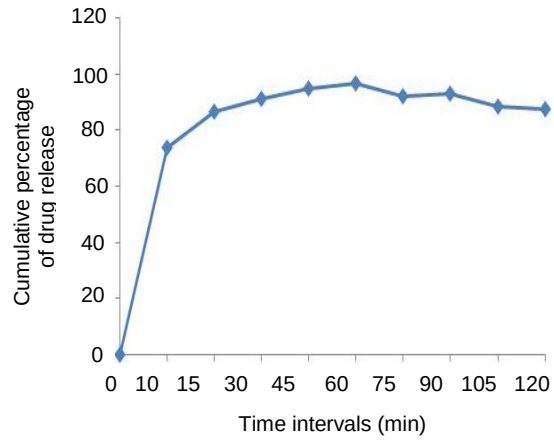


Fig 2. Cumulative percentage of drug release profile for *T. chebula* capsule (F4)

All formulations met the criteria of less than 30 minutes of capsule disintegration setin British Pharmacopoeia (2016).[19] The *in vitro* drug release behavior of the optimized capsules dosage form was

investigated at Department of Food and Drug Administration. The *in vitro* dissolution behavior was investigated for formula F4 as it gave the highest total phenol content (Fig. 2). The 500 mL of distilled water was used as the medium for *in vitro* dissolution study. The overall dissolution rate of capsule showing more than 70% of drug release from their dosage forms is clearly indicating the availability of drug at the site of absorption (Fig. 2)

*Conclusion*

The ethanolic extract of *T. chebula* Retz. fruit (Hpan-ga) from Magway Division showed the secondary metabolites except cyanogenic glycoside and no lethality of the mice was observed according to the acute toxicity study. Moreover, $IC_{50}$ value of viscous ethanolic extract showed 0.238 mg/mL. Thus, the ethanolic fruit extract having antioxidant activity was formulated as capsule dosage form. Among capsule formulations, the cap sule comprising MCC-loaded extract with 3% talc (F4) showed highest amount of total polyphenol, short disintegrating time and acceptable *in vitro* drug release. Therefore, the capsule comprising MCC-loaded extract granules (F4) was chosen as the reproducible capsule dosage form.

*Competing interests*

The authors declare that they have no competing interests.

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