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Extraction of Polyphenols from *Decalepis hamiltonii* Root: Optimization Of Batch Extraction Process Parameters.

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ABSTRACT

The extraction of total phenolic contents and total flavonoid contents of *Decalepis hamiltonii* roots was investigated at various experimental conditions. One factor at a time parameter optimization has been done. The experimental parameters studied were extraction temperature (30-60 ^{o}C), extraction time 30-240 min), extraction solvent (Aqueous solutions of ethanol, methanol, acetone, 100 % ethanol, 100% methanol and 100% acetone), ethanol concentration (20-100 %) and agitation speed (50-250 rpm). The best batch conditions for selected parameters were found to be 60 min extraction time, 150 rpm agitation speed, 60 ^{o}C temperature, 1:1 aqueous acetone solvent system and 60 % ethanol concentration and TPC and TFC content found to be 0.337 and 0.0422, 0.0213 and 0.067, 0.05 and 0.03, 0.057 and 0.103, 0.09 and 0.033 gm GAE/gm DHRP and gm RE/gm of DHRP respectively.

Keywords: *Decalepis hamiltonii,* Total Phenolic Content, Total Flavonoid Content, Batch Extraction.

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INTRODUCTION

Decalepis hamiltonii (swallow root) is a monogeneric climbing shrub endemic to the Deccan peninsula and forest areas of western ghats of India. Decalepis hamiltonii commonly called as maredu kommulu or barre sugandhi or maradu gaddalu or makali beru belonging to the family Asclepediaceae. The rhizome is largely used for pickling along with curd or limejuice, the roots have been used locally to stimulate the appetite and to relieve flatulence and act as a general tonic [1]. It is also useful as a blood purifier, preservative and as a source of bioinsecticide for stored food grains [2, 3]. It possess potent antioxidant properties, antiulcer, anti – inflammatory and antipyretic, gastro protective activities [4]. More recent studies on biologically active compounds in *Decalepis* have revealed the presence of a large number of flavonoids and phenolic compounds belonging to the family of polyphenols [3]. The role of the phenolics and flavonoids as natural antioxidants and free radical scavengers has attracted considerable recent interest due to their pharmacological behavior [5,6].

Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades [7]. Increased consumption of whole grains, fruits and vegetables reduce the risk of chronic diseases like cancer and heart diseases [8,9]. Epidemiological and *in vitro* studies indicate that these potential protective effects against different diseases are generally attributed to the presence of various functional components of food products containing phytochemicals such as phenolic and flavonoids compounds. These phytochemicals can be used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial agents [10]. Antioxidants minimize rancidity of foods, restrict the formation of toxic oxidation products, maintain nutritional quality and increase shelf life. Recently interest has been in-creased considerably in finding natural occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [8].

The aim of any extraction process should be, of course, to provide for the maximum yield of substances and of the highest quality (concentration of target compounds) and antioxidant power of the extracts. Extraction efficiency is influenced by various factors such as method of extraction, solvent type, solvent concentration, contact time, extraction temperature, solid to solvent ratio and particle size. [2, 11] Nevertheless, solvent type has a major importance in extraction efficiency. Solvent extraction is frequently used for isolation of antioxidant and extraction yield is dependent on the solvent and method of extraction, due to the different antioxidant potentials of compounds with different polarity [12]. The most broadly applied extraction procedure is solvent extraction using, extractants such as methanol, ethanol and acetone or mixtures of these with water for the recovery of a wide range of polyphenols of diverse phenolic structures [13]. Furthermore, the use of water in combination with other organic solvents contributes to the creation of a moderately polar medium that ensures the extraction of polyphenols [2].

The main objectives of this study were to optimize the batch extraction parameters such as extraction time, extraction temperature, agitation speed, different solvent system and ethanol concentration for extraction of polyphenols from *Decalepis hamiltonii* root.

MATERIALS AND METHODS

Sample preparation

The *Decalepis hamiltonii* roots were collected from Local Market, Malleswaram, Bangalore, India. The roots were thoroughly washed with tap water to remove adhered soil and manually cut into small pieces. Then, they were dried in shade for about 2-3 days. Once after the sample has been dried, the sample was ground to powder using a domestic grinder. The powder was then separated into different particle size fractions using a set of sieves in a laboratory sieve shaker (M/s Muhlenbau, Germany).

Chemicals and reagents

Aluminium chloride, Sodium hydroxide, Folin-Ciocalteau, Gallic acid, rutin was obtained from SDFCL. Sodium nitrate and Sodium bi-carbonate were purchased from Merck specialties private limited. All other chemicals and solvents used for the study were of analytical grade. The water used for the extraction and analysis was double distilled water.

Extraction of polyphenols from *Decalepis hamiltonii*

Extraction of polyphenols was performed at 5 different operating conditions:

Effect of extraction time

Effect of extraction time on extraction of Polyphenols was carried out at different time intervals (30, 40, 50, 60, 70 and 80 min) and by fixing temperature (30^0C), agitation speed (150 rpm), solvent (distilled water) and solid/solvent ratio (0.5 g in 25 ml solvent)

Effect of agitation speed

Effect of agitation speed on extraction of polyphenols was carried out at different speed (50, 100, 150, 200 and 250 rpm) and by fixing temperature (30^0C), time (60 min), solvent (distilled water) and solid/solvent ratio (0.5 g in 25 ml solvent)

Effect of extraction temperature

Effect of temperature on extraction of polyphenols was carried out at different temperatures (30, 40, 50, 60, 70 and 80^0C) and by fixing speed(150 rpm), time (60 min), solvent (distilled water) and solid/solvent ratio (0.5 g in 25 ml solvent).

Effect of solvent system

Effect of different solvents on extraction of polyphenols was carried by using different solvents (ethanol, methanol, acetone, water, 1:1 aqueous solutions of acetone, ethanol and methonol) and by fixing temperature (30^0C), time (60 min), agitation speed (150 rpm) and solid/solvent ratio (0.5 g in 25 ml solvent).

Effect of ethanol concentration

Effect of ethanol concentration on extraction of polyphenols was carried by using different ethanol concentration (20%, 40%, 60%, 80% and 100%) and by fixing temperature(30^0C), time (60 min), agitation speed (150 rpm) and solid/solvent ratio (0.5 g in 25 ml solvent).

Spectrophotometric determination of total polyphenols:

Total phenolic content (TPC) assay was performed using Folin-Ciocalteu reagent (FCR) method [15]. Different volume of extracts was mixed with previously diluted Folin-Ciocalteu for 1:10 v/v with water and 4 ml (75g/l) of sodium carbonate. Absorbance was taken using UV-Spectrophotometer against blank at 725 nm after incubation for 30 min. Measurements were calibrated using a gallic acid standard curve. The TPC of the extracts were expressed as gallic acid equivalent (GAE) g/100 g of *Decalepis hamiltonii* root powder [5].

The total flavonoid contents were measured by a Aluminum chloride method. An aliquot of extract was appropriately diluted and mixed with 2ml of distilled water. At zero time, 0.15 ml 5% sodium nitrite was added to the flask. After 5 min, 0.15 ml of 10% aluminium chloride was added. At 6 min, 2 ml of 4% sodium hydroxide was added to the mixture. Instantly, volume of the mixture was made upto 5ml and thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus a blank. Rutin was used as standard for the calibration curve. Total flavonoid content of the extracts and fractions were expressed as g rutin equivalents (RE) per gram of *Decalepis hamiltonii* root powder [10].

Result Analysis

All the experiments and assays were carried out in triplicates and mean values are presented in the graphs.

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RESULTS AND DISCUSSION

Effect of extraction time on extraction of Polyphenols

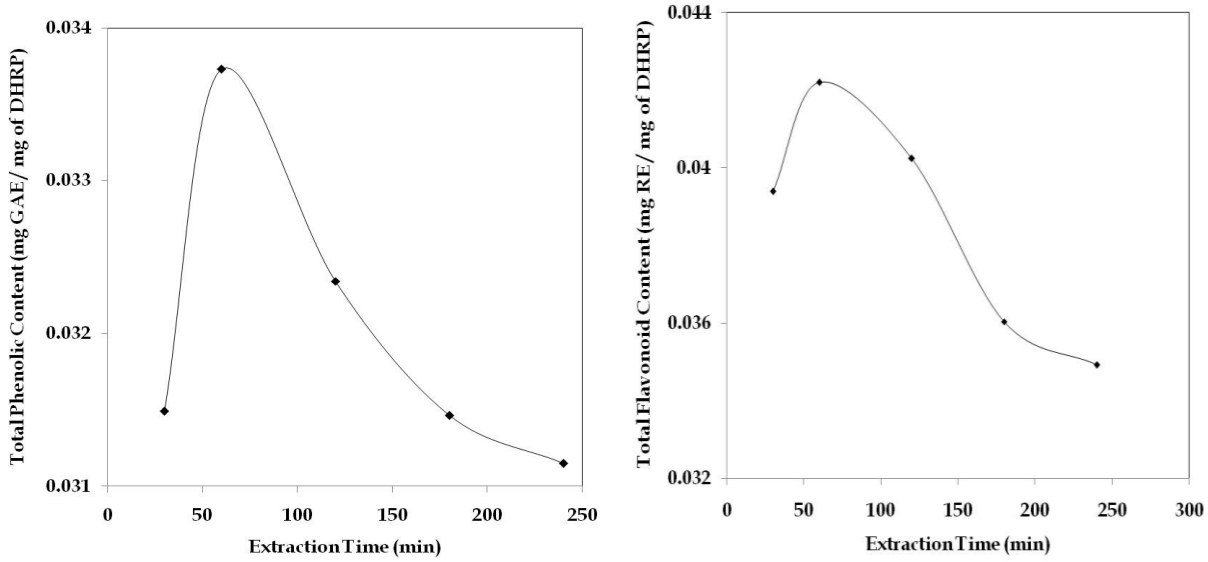

Figure 1: Effect of extraction time on polyphenols (a) Total Phenolic Content (b) Total Flavonoid Content of *Decalepis hamiltonii.*

Samples were extracted using the double distilled water. The extraction conducted by varying the extraction time from 30 to 240 min while fixing the extraction temperature and speed at 30^0C and 150rpm respectively. The best extraction time was found to be 60 min according to the value of TPC (mg GAE/100 g root powder) and TFC (mg RE/100 g root powder). The extraction of TPC and TFC was found to increase from 0.0315 mg to 0.0338 mg and 0.038 mg to 0.043 mg respectively with an increase in extraction time from 30 to 60 min (Fig. 1). The increase in extraction up to a certain extent may be attributed to the efficient contact between solvent and particles. Beyond 60 min, the extraction of TPC and TFC was found to decrease up to 0.0311 mg and 0.034 mg respectively. The prolonged stirring might have resulted in degradation of polyphenols.

Effect of agitation speed on extraction of Polyphenols

The speed of mixing is a key factor in the rate of extraction of polyphenols. The effect of stirring speed was investigated in the range of 50–250 rpm in order to obtain optimal speed with effective extraction of polyphenols as shown in Fig. 2. When the mixing speed was increased from 50 to 150 rpm, the extraction of TPC and TFC was found to increase from 0.01 mg to 0.07 mg and from 0.01 mg to 0.022 mg, respectively. Further increase in stirring rate above 150 rpm resulted in decrease in both the extraction of TPC and TFC. This may be attributed to the fact that increasing stirring speed up to 150 rpm resulted in the proper mixing and close contact between particles and solvent. However, stirring speeds >150 rpm increase the vigorous mixing which spins round the mixture so fast leading to the formation of vortex with less contact between particles and solvent resulting in less extraction.

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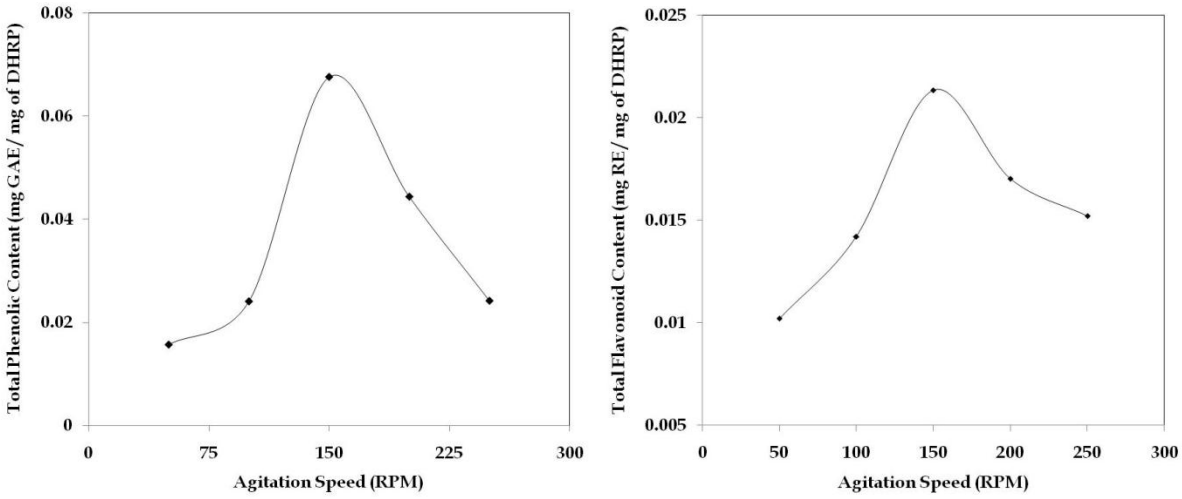

Figure 2: Effect of agitation speed on polyphenols (a) Total Phenolic Content (b) Total Flavonoid Content of *Decalepis hamiltonii.*

Effect of Temperature on extraction of Polyphenols

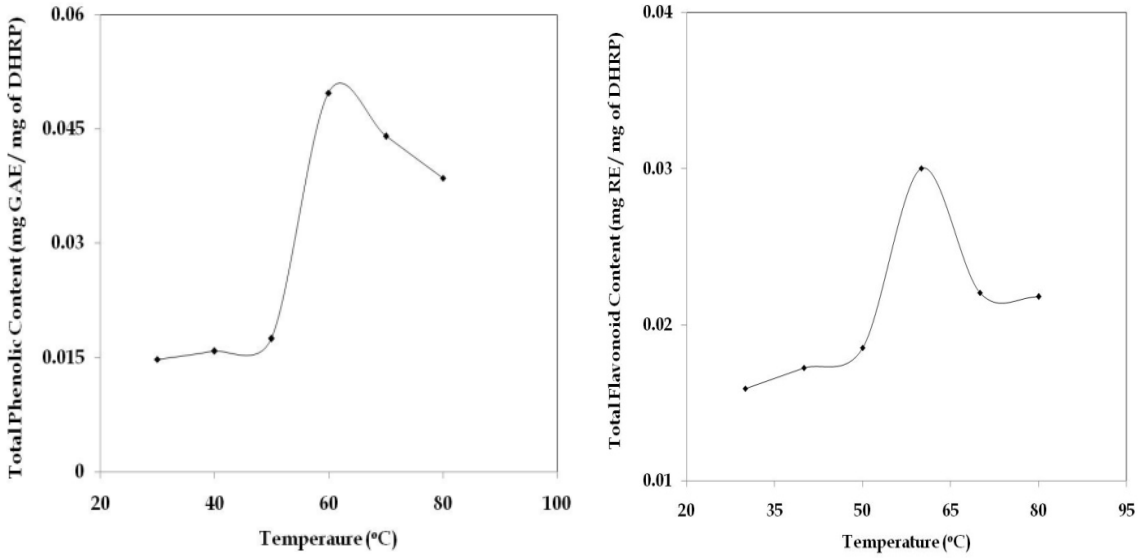

Figure 3: Effect of Temperature on polyphenols (a) Total Phenolic Content (b) Total Flavonoid Content of *Decalepis hamiltonii.*

Powdered *Decalepis hamiltonii* roots were extracted at six temperatures, 30, 40, 50, 60, 70 and 80 °C. The results in Fig. 3 showed that polyphenol extracts obtained at the extraction temperature of 60°C contained higher TPC and TFC. Increasing the extraction temperature from 30 to 60 °C resulted in increase in TPC and TFC. This effect of temperature confirms an increase in solubility and hydrolytic reactions. The use of higher temperatures increases the capacity of water to solubilize polyphenols. At specific temperature (60°C), a maximum yield of polyphenols was obtained. Temperature above 60 ^{o}C probably caused a decrease in the

extraction yield due to possible degradation of phenolic compounds, caused by hydrolysis, internal redox reactions and polymerization [14]. According to Durling *et al*. [15] an increase in temperature resulted in the increased extract yields, but at a higher temperature (63°C) the yield was lower, because more inactive compounds were extracted from source. Thermally labile compounds can also be extracted with minimal damage by using low temperatures.

Effect of different solvents on extraction of Polyphenols

The polyphenols were extracted with methanol (50:50, v/v), ethanol (50:50, v/v), acetone (50:50, v/v), water, ethanol, methanol and acetone. The extraction capability of these different solvents for extraction phenolic and flavonoid compounds are presented in Fig. 4. The amount of extract recovered by employing aqueous acetone as a solvent was significantly higher than when other solvent systems were used under the same extraction. These results indicate the importance of choice of solvent in quantification of different components of the extracts. Acetone and methanol are not recommended to use in food and in the manufacture of drug substances because of their unacceptable toxicity or their deleterious environmental effect. So ethanol which is categorized under GRAS (Generally Recognized as Safe) would be preferable in view of the application in food system and pharmaceuticals. Ethanol was chosen as the extraction solvent for the next experiments.

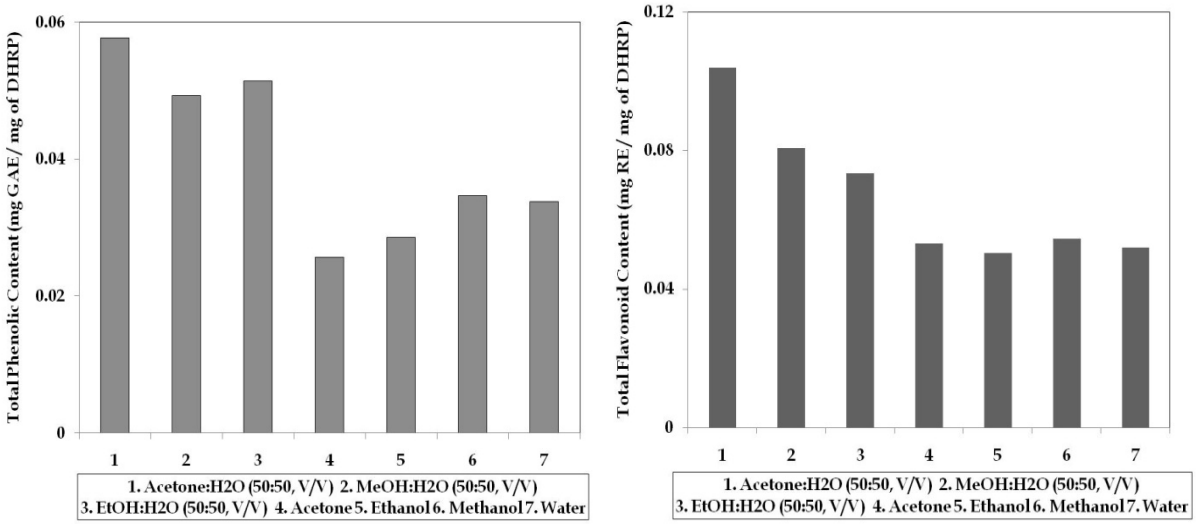

Figure 4: Effect of solvents on polyphenols (a) Total Phenolic Content (b) Total Flavonoid Content of *Decalepis hamiltonii.*

Effect of Ethanol concentration on extraction of Polyphenols

The effects of different ethanol concentration (20%, 40%, 60%, 80% and 100%) on extraction of phenolic and flavonoid compounds from *Decalepis hamiltonii* roots were shown in Fig. 5. TPC and TFC increased with the increment of the ethanol concentration up to 60% followed by a reduction at 100%. Similarly, Cacace and Mazza [16] revealed that maximum total phenolics in black currants extracts was obtained at about 60% ethanol followed by a

decrease with further increase in concentration. Chaalal M *et al* [2] also found that increasing in ethanol concentration beyond 70% will dramatically reduced the amount of phenolics extracted from peanut skins. Remarkable drops in TPC and TFC at 100% ethanol revealed that absolute solvent do not ensure a good recovery of phenolic compounds as compared to aqueous ethanol. Different authors [17,18,19] suggested that ethanol reduces the dielectric constant of the solvent, thus increasing the diffusion of the bioactive molecules with the solvent. However, highly pure organic solvents such as 100% ethanol, could dehydrate the vegetable cells, making difficult the diffusion of polyphenols from the plant material to the extracting liquid. Thus, ethanol concentration of 60% was found to be maximum and effective for the extraction.

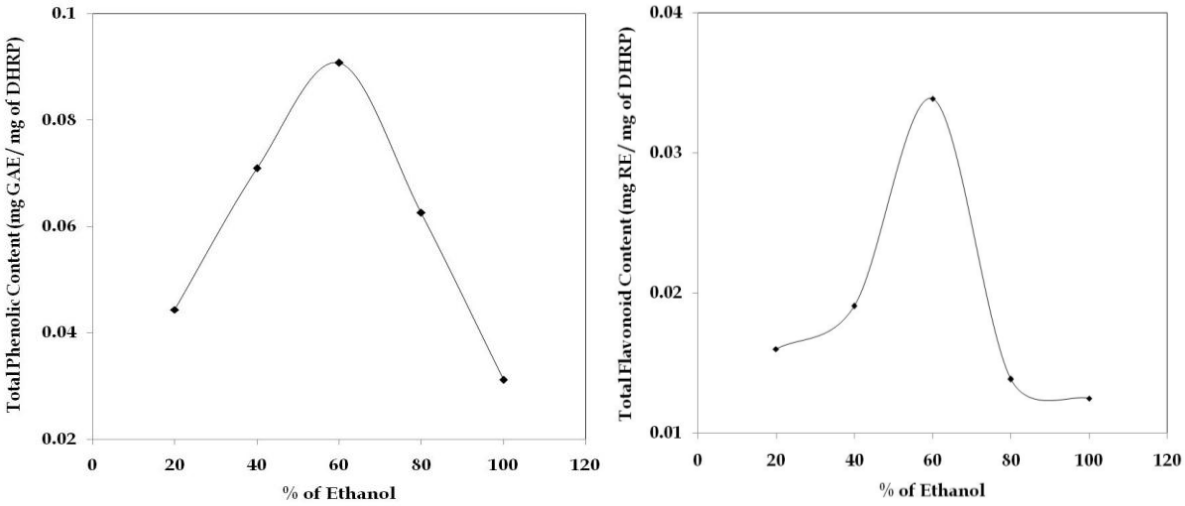

Figure 5: Effect of concentration on polyphenols (a) Total Phenolic Content (b) Total Flavonoid Content of *Decalepis hamiltonii.*

CONCLUSION

This study reflects the importance of controlling the studied extraction conditions (time, temperature, agitation speed, selection of solvent and % ethanol) to obtain an extract with the highest polyphenols content. It can be concluded that the optimum conditions for maximum polyphenols extraction were as follows: ethanol concentration, 60%; agitation speed, 150 rpm; temperature, 60^0C and extraction time, 60 min. The results from extraction showed that TPC and TFC of *Decalepis hamiltonii* roots were most affected by ethanol concentration followed by extraction temperature and extraction time. Further works may carry out under the optimum conditions to elucidate the identity of phenolic compounds responsible for the antioxidant properties of *Decalepis hamiltonii* roots.

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