REVIEW AND CALCULATION OF ACTIVITY CO EFFICIENT OF TEA POLYPHENOLS AND ITS SOLUBILITY IN DIFFERENT SOLVENTS

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Abstract

Tea, one of the most popular beverages, has been consumed for thousands of years for their flavors and health benefits. Polyphenols are the flavanoid and phenolic acids in tea that contribute to health benefits. There are more than 45 polyphenols in tea. In this review, the structure, health benefits and suitable solvent selection for extraction of these polyphenols is covered. Further the calculation of activity coefficient of tea polyphenol is also done. This study helps us to understand the suitability of a solvent and its temperature dependency in its extraction process from tea leaves.

Keywords: *Tea, polyphenols, solvents.*

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1. INTRODUCTION

The search for natural antioxidative compounds from plant materials has drawn lot of attention in the past few decades. Tea, one of the most popular beverages, has been consumed for thousands of years for their flavors and health benefits. Tea is produced by brewing the dried leaves and buds of the plant *Camellia sinensis,* which was first cultivated in China and then in Japan. During the 15th to 17th centuries, commercial cultivation gradually expanded to Indonesia and then to the Indian subcontinent, including what is now in Sri Lanka [18]. Tea is now second only to water in worldwide consumption. Annual production of about 1.8 million tons of dried leaf provides world per capita consumption of 40 L of beverages. [19].

The two major types of tea are unfermented tea (Green tea) and fully fermented tea (Black tea). Polyphenols are the flavanoids and phenolic acids in tea that contribute to health benefits. There are more than 45 polyphenols in tea [1]. Catechin (also called as epicatechin) is a major class of tea polyphenols that has novel characteristics as an antioxidant. It is also a neutraceutical compound which constitutes about 30-40% by dry weight of tea [2]. The polyphenolic content is higher in an unfermented green tea. Green tea production avoids oxidation of polyphenols. In contrast, black tea is produced by promoting enzymatic oxidation of tea polyphenols. Referring to table 1 we know that the structure of polyphenols have several -OH groups bonded to aromatic rings enables the molecule to scavenge free radicals which causes the antioxidant activity by preventing damage on the cellular level [3]. The content and composition of polyphenols vary among different types of tea depending on the individual mode of preparation and degree of fermentation [4].

Here we review the polyphenolic chemistry of tea along with its structure, health benefits, its solvent selection and its temperature dependency necessary for its extraction from tea leaves.

2. STRUCTURES[12-16]

The family of green tea catechins consists of 21 different molecules, but the four epimers in Figure 1, namely, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECg), and epigallocatechin gallate (EGCg), account for 93% (dw) of them, with epigallocatechin gallate having the highest concentration, 46% (dw).

3. HEALTH BENEFITS

Tea polyphenols have good biological and pharmacological activities like lipid peroxidation, anti oxidative suppression of low density lipoproteins, anti-mutagenecity, anti-HIV effect, anti-allergic effect, DNA cleavage, anti-topoisomerase (1,2) , DNA scission etc [5]. There are limited evidence to suggest benefits of tea consumption to reduce body weight and fatness. Effect of fermentation process on tea has its contribution to apoptosis and cell cycle arrest of gastric cancer cells [6]. Research shows that catechins are more involved in such cell cycle arrest mechanism. They have also proved to inhibit activities of sucrase and alpha glycosidase [7]. Through experiments it is found that there is a certain threshold concentration for catechin to suppress the plasma glucose levels. Results do explain that the possible indigestibility of catechin would not cause any malnutrition. A lot of vitro studies, animal and human research demonstrates tea as anti-bacterial, anti-viral, and also a high potential to protect against atherosclerosis and cardio vascular diseases [8]. The therapeutic effects of green tea and its catechin phenolics is examined in microbial and mammalian cell systems and is found to suppress formation of mutagenic and carcinogenic heterocyclic amines in cooked food thereby adding to its health benefits [8].

4. MATERIALS AND METHODS

Leaves of Camellia sinensis , Solvents like water, methanol, ethanol, propanol, acetone, methyl acetate, ethyl acetate, dichloromethane, chloroform, benzene, and toluene were tried out.

Methods used for extraction	**Comments**
Enzymatic extraction [3]	• High content of polyphenols obtained but antioxidant property decreases • Used in grape wine polyphenol extraction but not in tea.
Using Resins [20]	• It is a non toxic method but not economical • Used in Tea polyphenol extraction
Molecular distillation and spray drying [21]	• Efficient extraction occurs but the maintenance of the optimum conditions are difficult.

5. SOLVENT SELECTION FOR EXTRACTION

The yield and antioxidant activity of natural extracts are dependent on solvent and temperature used for extraction. For example absolute methanol is suitable to extract tea polyphenols, 50% acetone is used to extract wheat polyphenols, water or salt solutions at neutral pH or 20% organic solvent in water are efficient solvents to remove the polyphenols in sunflower seed, while water and ethanol mixture is used to extract chestnut tree wood polyphenols. Tea polyphenols are highly extracted in aqueous acetone. Its solubility in water is low when compared to its solubility in organic solvents [9,10]. On the other hand, chlorogenic acid, the major polyphenol in sunflower seed has high solubility in aqueous solution like water and very less in organic solvents. It is because of the strengthening of hydrogen bonds in organic solvents and vice versa in water. Also the basicity and ionisation of sunflower polyphenols are high in water which accounts for their high solubility [11]. This shows that solvent selection depends mainly on the type of polyphenols and its complex structure present which varies in every plant material.

6. CALCULATION OF LOG P VALUES TO PREDICT SOLUBILITY

The calculated log P value for a compound in water vs. a simple organic compound like octanol or hexane can provide a guideline for predicting its solubility characteristics in other aqueous and organic solvents. For example, the value of catechin 1.5 indicates that its ratio is 15:1 in organic : aqueous compounds. This proves that it is hydrophobic and requires an organic solvent.

The log P value for a compound is the logarithm (base 10) of the partition co-efficient (P), which is defined as the ratio of the compounds organic(oil) to aqueous phase concentrations [17]

Partition co-efficient

(P)=[organic] / [aqueous]

where, [ ] is the compounds concentration in solvent phase. Using chemdraw software the structures of the polyphenols were drawn and the log p values for some of the major polyphenols were found. The calculated log p value provides a guideline to predict the solubility characteristics of the polyphenols in aqueous and organic solvents.

Polyphenols	**Log P value**
catechin	1.5
epicatechin	1.5
gallocatechin	1.11
epigallocatechin	1.11

Thus it serves the purpose of elementary prediction, whether it requires an organic or an aqueous solvent.

7. METHODOLOGY

Modified UNIFAC (Dortmund) method [18] was employed to calculate the activity coefficient of tea polyphenols. Activity coefficients are usually modeled by GE models such as the UNIFAC (universal functional activity coefficient) model. This model has proven to be successful for small biomolecules. The UNIFAC model is considered to be predictive, although not entirely, because it does not require any experimentation for the calculation of properties but uses parameters that have already been regressed from a vast database of equilibria. In the modified UNIFAC (Dortmund) model the activity coefficient is the sum of a combinatorial and a residual part:

$$\ln \gamma_i = \ln \gamma_i^C + \ln \gamma_i^R \quad - (1)$$

Combinatorial part was changed in an empirical way to make it possible to deal with compounds which vary in size.

$$\ln \gamma_i^C = 1 - V_i' + \ln V_i' - 5q_i \left(1 - \frac{V_i}{F_i} + ln\left(\frac{V_i}{F_i}\right)\right) \quad - (2)$$

The parameter Vi' can be calculated by using the relative Van der Waals volumes $\boldsymbol{R_k}$ of the different groups.

$$V_i' = r_i^{\frac{3}{4}} / \sum_j x_j \, r^{\frac{3}{4}}{}_j$$

All the other parameters are calculated in the same way as in the original UNIFAC model.

$$V_i = r_i \,/ \sum_j x_j \, r_j$$

$$r_i = \sum v^{(i)}{}_k \, R_k$$

$$F_i = q_i \,/ \sum_j x_j \, q_j$$

$$q_i = \sum v^{(i)}{}_k \, Q_k$$

(**R and Q values are given in table 2**)
Where,
x = mole fraction
R = volume parameter of group
Q = surface area parameter of group
υ = frequency of group
Where residual part is calculated by,

$$\ln \gamma_i^R = \sum_k v^{(i)}{}_k \left(\ln \Gamma_k - \ln \Gamma^{(i)}{}_k\right) \; - (3)$$

$$\ln \Gamma_k = Q_k \left(1 - \ln\left(\sum_m \theta_m \, \psi_{km}\right) - \sum_m (\theta_m \, \psi_{km} / \sum_n \theta_n \, \psi_{nm})\right)$$

whereby the group area fraction θ_m group mole fraction Xm are given by,

$$\theta_m = Q_m X_m / \sum_n Q_n \, X_n$$

$$X_m = \sum_j v^{(i)}{}_m \, x_j / (\sum_j \sum_n v^{(j)}{}_n \, x_j)$$

In comparison to original UNIFAC method, only the van der Waals properties were changed slightly, at the same time temperature dependant parameters were introduced to permit a better description of the real behavior (activity coefficient) as a function of temperature.

$$\psi_{nm} = \exp\left(-(a_{nm} + b_{nm}T + c_{nm}T^2/T)\right)$$

Where,
Ψ=interaction parameter

8. RESULTS AND DISCUSSIONS

The activity coefficient of all the polyphenols was calculated in solvents: water, methanol, ethanol, propanol, acetone, methyl acetate, ethyl acetate, dichloromethane, chloroform, benzene, and toluene. Some of the interaction parameter values were not available for solvents like water, dichloromethane and chloroform in Modified UNIFAC (Dortmund) method. So only these solvents were tried by UNIFAC LLE and VLE [19, 20]. But their gamma values were found to be increasing with increase in temperature which was not acceptable. So these solvents were not used for further studies. Methyl acetate, ethyl acetate and acetone have all their interaction parameters available in Modified UNIFAC (Dortmund), but, their gamma values showed increasing trend with temperature (**Figure 1**). Since the gamma value increases negligibly with temperature in acetone it was considered for further calculations. When methanol was considered as a single group (as per Modified UNIFAC (Dortmund)) it had the increasing trend of gamma values, while it decreased when considered as a combination of methyl and OH group. Hence, methanol was splitted as methyl and OH group and used for calculations. But its predictions may not be reliable. All predictions were made at polyphenol mole fraction of 0.1

From **Figure 1**, methanol, ethanol, propanol, butanol, the gamma value increases with increase in Carbon chain. This is because of the effect of steric hinderence formed by the increase in Carbon chain length of the solvents. Comparing values of ethanol, acetone (having 2 Carbon) the order of gamma value is OH>CO in Modified UNIFAC (Dortmund) method. But since acetone predictions were not reliable this cannot be interpreted. Of all the solvents tried with catechin methanol showed the lowest gamma value. But as said before methanol cannot be relied. So the next best solvent was ethanol. From the solubility values available in literature [21], the predicted values were compared using solid liquid equilibrium theory. From table it is observed that ethanol gave twice the values of experimental values and ethyl acetate gave almost the accurate value but only at 298K.

SOLVENTS	SOLUBILITY USING MODIFIED UNIFAC (DORTMUND) METHOD(g/L)	SOLUBILITY EXPERIMENTAL VALUES(g/L)
METHANOL	825.83	-
ETHANOL	482.7	291.84
PROPANOL	205.37	-
BUTANOL	85.64	-
ETHYL ACETATE	185.18	190.11

CONCLUSIONS

- Tea polyphenols have many health benefits and has a major role to play in pharmacy.
- So a suitable solvent selection for their extraction is the ultimate aim of this paper.
- The structures of all the polyphenols were studied, and they were used to predict the their activity coefficient values in different solvents at different temperatures.
- From all the methods tried, Modified UNIFAC (Dortmund) method was more applicable for solubility calculations.
- This method worked well with solvents with alcoholic group and non-polar solvents and to a lesser extent with acetone.
- Among the solvents methanol had the highest solubility but since methanol did not work out in Modified UNIFAC (Dortmund) method when tried as a single group it cannot be supported strongly.
- So the next best solvent is ethanol.
- Still there are many solvents to be tried out at different temperatures to find their effect on solubility of tea polyphenols.

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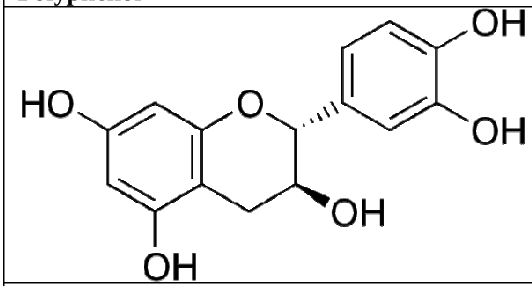
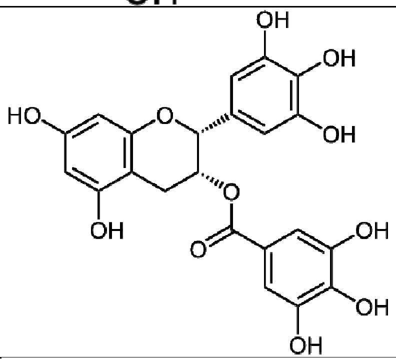
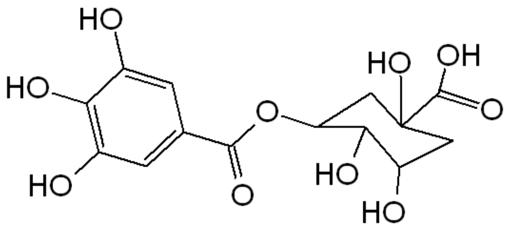
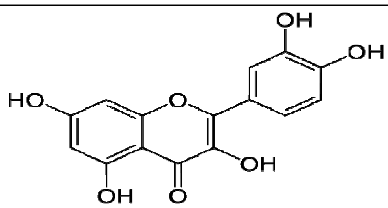
Table:1 Structures of major tea polyphenols [12-16]

Polyphenol	**Structure**
	**Epicatechin**
	**Epicatechin Gallate**
	**Theogallin**
	**Quercitin**

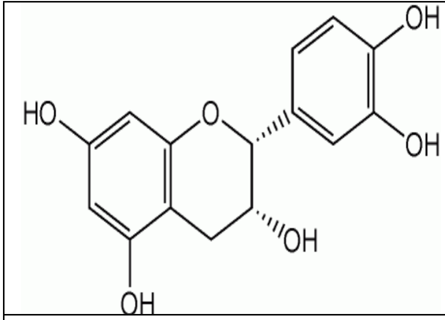
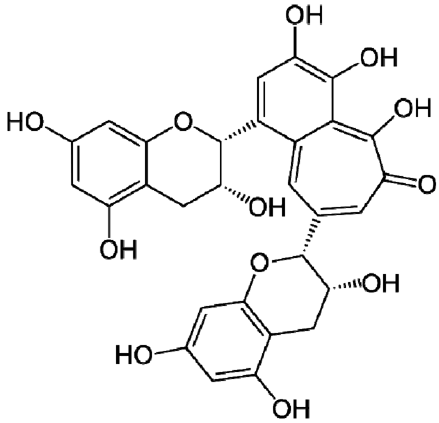
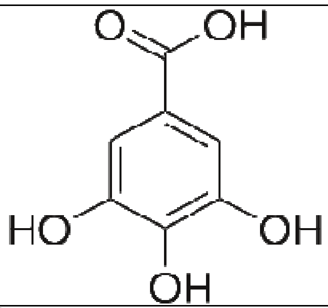
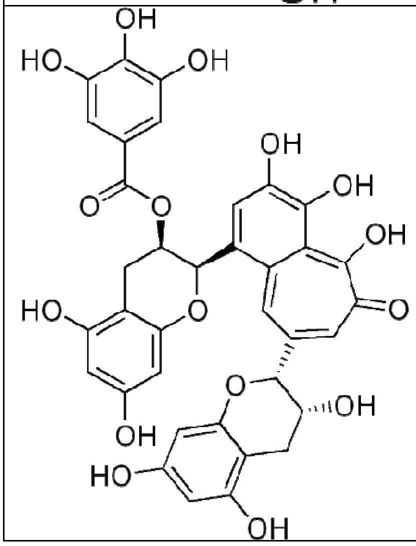
	**Proanthocyanidins**
	**Theaflavin**
	**Gallic acid**
	**Theaflavin gallate**

Table:2 Surface area and volume parameters: [18]

Groups	R	Q
CH2=C	1.2832	0.8962
ACH	0.3763	0.4321
AC	0.3763	0.2113
ACCH2	0.91	0.7962
ACCH	0.91	0.3769
OH(S)	1.063	0.8663
OH(T)	0.6895	0.8345
ACOH	1.08	0.975
CCOO	2.535	3.3912
CHO	1.1434	0.8968
c CH2	0.7136	0.8635
c CH	0.3479	0.1071
c C	0.347	0
CH3OH	1.7048	1.67
COOH	0.8	0.9215

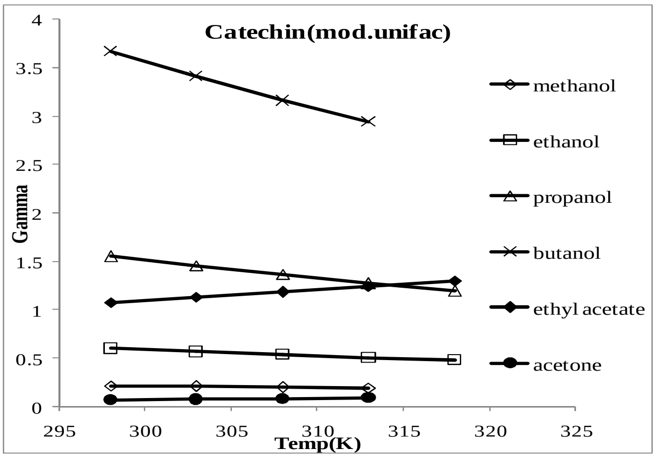


Figure1