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PHYSICOCHEMICAL CHARACTERIZATION OF *GARCINIA GUMMI-GUTTA* (L.) ROBS. SEED OIL

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ABSTRACT: The objective of the present study involves the evaluation of physicochemical characteristics of the oil extracted from *Garcinia gummi-gutta* seeds. The seed oil is yellowish brown coloured without any characteristic odour and it is solid at room temperature with a melting point of 39.8°C and 9.27% of moisture content. The refractive index of the seed oil was determined as 1.460. *G. gummi-gutta* seed oil is soluble in non-polar solvents and its pH was determined as 4.69. The parameters such as acid value, saponification value, iodine value, peroxide value and ester value of *G. gummi-gutta* seed oil were determined by standard methods and they were 3.07±0.18 mg KOH/g oil, 185.94±0.32 mg KOH/g oil, 43.86±0.24 g/100 g oil, 3.47±0.18 meq/kg and 182.87±0.55 respectively. The results showed that the acid value, saponification value and peroxide value of *G. gummi-gutta* seed oil are within the range of edible oils. The present study points out that *G. gummi-gutta* seeds can be used as an alternate source for edible oil. Detailed nutritional and anti-nutritional studies of the seed oil are warranted to confirm its edibility.
Key words: Physicochemical characteristics, acid value, saponification value, peroxide value

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INTRODUCTION

Garcinia gummi-gutta (L.) Robs. commonly called 'Malabar Tamarind' (Family: Clusiaceae) and locally known as "Kudampuli". It is an indigenous, tropical, under exploited tree found distributed in the evergreen forests of Western Ghats from Konkan to Kerala and Shola forests of Nilgiri hills etc. It is also seen in the home gardens of Kerala and the acidic fruit rind is used in curries as a souring condiment (Abraham et al., 2006). It is a small to medium sized tree that attains an average height of 25 m and the branches are horizontal or drooping with dark, smooth and lactiferous bark (Lim, 2012). The leaves of the plant are simple, entire, petiolate, opposite, dark green, elliptic to obovate and glabrous. Male and female trees are separate and male trees have tetramerous flowers in axillary umbellate clusters whereas female flowers are tetramerous, terminal or axillary and solitary or 1-3 fascicles. They are seen usually red, but some trees have yellow flowers. The fruits are fleshy, globose, sub-globose to ovoid berry, green turning yellow or reddish colour when ripe, having 6-8 grooves. The seeds are 6-13, surrounded by a succulent aril. Flowering season of the plant is during December to February and fruiting season from March to June (Selvaraj and Avadhani, 2013).

Garcinia gummi-gutta is known for its medicinal values in Ayurveda and used against arthritis, uterine diseases and ulcers. It is traditionally used for the treatment of constipation, edema, delayed menstruation, hemorrhoids, fever, dysentery, diarrhoea and an established plant for reducing body weight (Madappa and Bopaiah, 2012). The plant parts such as fruits, bark, leaves etc. have been scientifically evaluated for various therapeutic effects. The medicinal importance of the plant is due to the secondary metabolites such as alkaloids, glycosides, volatile oils, tannins, flavonoids, phenolic compounds etc. and the main component of *G. gummi-gutta* fruits is hydroxy citric acid (Kim et al., 2011). The dried seeds yield edible fat commonly known as 'seed butter', due to its solid state in room temperature, and it is a rich source of protein and fat. According to Abraham et al. (2006), the seeds of *G. gummi-gutta* are a waste product of post-harvest operations. The seed oil is traditionally used for the treatment of skin diseases (Sharmila et al., 2015). *Garcinia indica*, a closely related species of *G. gummi-gutta* yield seed butter commonly known as 'Kokum butter' is used by the local people to improve digestion, to cure dysentery, pain, heart complaints, piles and tumour. It also exhibited anathematic and cardio-tonic properties. It can be directly used for frying and also in chocolate, confectionary and cosmetic industries (Maheshwari and Reddy, 2005).

Fats and oils are hydrophobic hydrocarbon molecules and they are the storage form of energy in plants, found much abundant in seeds. It is the second largest energy source for living cells and belongs to the group of compounds known as lipids (Jain et al., 2005). Fats and oils are the important sources or raw materials for the development of edible and non-edible products. Improvement of fats and oils is the main strategy for the product development and the improvements can be classified in to three categories such as application development, triglyceride replication and analytical development (O'Brien, 2003). In India, there is a shortage of edible oil production to meet the current requirement and India is the third largest consumer of edible oils with a domestic consumption of 12.5 million tons, whereas the domestic production of edible oil is only 8.2 million tons. Under these circumstances, the country needs to double the oil seeds production to meet the future requirement. Due to socioeconomic and cultural constraints of oil seed farmers, the strategies and technologies developed by the oil seed research network have not been completed successfully (Choudhary et al., 2014). Hence, to narrow down the gap between production and requirement of edible oils, there is a need for the exploration of other oil seed sources (Cardoso et al., 2012).

There is an increasing interest among the researchers on seed oil analysis becauve of the extensive demands for oils, both for human consumption and for industrial applications (Kyari, 2008). It is necessary to assess the nutritional value and oil quality of fats and oils by physicochemical studies for the development of functional food or nutraceutical (O'Brien, 2003). Physicochemical characteristics depend on the nature of the oil and it is determined to know the purity, quality and identification of the oils. The present study deals with the determination of the physicochemical characteristics of *G. gummi-gutta* seed oil and such information may expand the scope of knowledge on the proper utilization and quality of the seed oil for different purposes.

MATERIALS AND METHODS

Extraction of the seed oil

The seeds of *Garcinia gummi-gutta* were collected from the homesteads of Mala locality, Thrissur District, Kerala and it was authenticated by the plant taxonomist. A voucher specimen (PCASH 01 dated 15/05/2017) was deposited in the College Herbarium for future reference. The seeds were separated from the succulent aril of fresh ripen fruits, washed thoroughly and dried. The seed kernels were separated from the seed coat and second level of drying was carried out. 100 g of the kernels were washed in hot water to remove the impurities and they were slightly roasted in gentle heat in a pan and grinded, boiled with 1 L distilled water in an open container for 2 to 3 h. The oily upper layer was separated and decanted in to a clean vessel and allowed to evaporate water content. Then, the seed oil or the seed butter was stored in dry, air tight containers.

Chemicals

Sodium thiosulphate, starch, potassium iodide (KI), Hanus Iodine solution, glacial acetic acid, potassium hydroxide (KOH) and phenolphthalein were purchased form SD Fine-Chem. Ltd., Mumbai. All the other chemicals used for the experiments were of analytical reagent grade.

Analysis of physical properties

The physical properties such as colour, odour and physical state of the seed oil were determined by sensory evaluation. The percentage oil content was determined from the extraction and the percentage moisture content was determined by the oven dry method. The refractive index of the seed oil was determined by using Abbe refractometer. The melting point of the oil sample was also determined by standard method (Ibeto et al., 2012).

Analysis of chemical properties

The chemical characteristics such as solubility, pH and ash content (%) of the seed oil were determined. The pH was determined by using pH meter (Analab Scientific, India) and ash content (%) by heating the oil sample to dryness in muffle furnace (Rotek Instruments, India). Other chemical properties like free fatty acids, acid value, saponification value, iodine value, peroxide value and ester value were determined by titrimetry according to the following standard methods.

Estimation of free fatty acids and acid value

2 g of melted oil sample was dissolved in 50 ml of the neutral solvent containing 25 ml ether, 25 ml of 95% alcohol, 1 ml of 1% phenolphthalein and 0.1 N KOH. A few drops of phenolphthalein was added and titrated against 0.1 N KOH, shaking constantly until a pink colour which persisted for 15 sec (Cox and Pearson, 1962). The acid value (mg KOH/g) was calculated from the following equation:

$$\text{Acid value (mg KOH/g)} = \frac{\text{Titre value} \times \text{Normality of KOH} \times 56.1}{\text{Weight of the sample (g)}}$$

The free fatty acid was estimated as oleic acid using the equation:
1 ml 0.1 N KOH = 0.028 g oleic acid

Determination of saponification value

The completely moisture free, melted and filtered oil sample (2 g) was added to 50 ml of alcoholic KOH from burette, in a conical flask by allowing it to drain for a definite period of time. A blank was also prepared by taking only 50 ml of alcoholic KOH in a conical flask, allowing it to drain at the same duration of time. Air condensers were connected to the flasks and boiled for about 1 h. After the flask and condenser get cooled, rinse down the inside of the condenser with distilled water and then removed the condenser, 1 ml of phenolphthalein indicator was added and titrated against 0.5 N HCl until the pink colour just disappeared (Horowitz, 1975). The saponification value was calculated from the equation:

$$\text{Saponification value} = \frac{28.05 \times (\text{titre value of blank} - \text{titre value of sample})}{\text{Weight of sample (g)}}$$

Determination of iodine value

0.5 g of the oil sample was taken into an iodine flask and dissolved in 10 ml of chloroform and 25 ml of the Hanus iodine solution using a pipette, draining it in a definite time. This mixture was allowed to stand in dark for exactly 30 min with occasional shaking. 10 ml of 15% KI was added, shaking thoroughly and 100 ml of freshly boiled and cooled water, washing down any free iodine on the stopper. It was titrated against 0.1 N sodium thiosulphate until yellow solution turned almost colourless. Then a few drops of starch was added as indicator and titrated until the blue colour completely disappeared. Towards the end of titration, stopper the flask and shook vigorously so that any iodine remaining in solution in $CHCl_3$ may be taken up by KI solution. A blank was also prepared without the sample (Horowitz, 1975). The iodine value was calculated from the following equation:

$$\text{Iodine value} = \frac{(B - S) \times N \times 12.69}{\text{g sample}}$$

Where, B = ml thiosulphate for blank; S = ml thiosulphate for sample; N = normality of thiosulphate solution.

Determination of peroxide value

1 g of the oil sample was weighed into a clean dry boiling tube and 1 g of powdered KI and 20 ml of solvent mixture containing two volumes of glacial acetic acid and one volume of chloroform was added. The tube was placed in boiling water so that the liquid boiled within 30 sec and allowed to boil vigorously for not more than 30 sec. The contents were transferred quickly to a conical flask containing 20 ml of 5% KI solution, washed the tubes twice with 25 ml water each time and collected into the conical flask. It was titrated against N/500 sodium thiosulphate ($Na_2S_2O_3$) solution until yellow colour almost disappeared. Then 0.5 ml of starch solution was added, vigorously shaken and titrated till the blue colour just disappears. A blank was also prepared without the sample (Cox and Pearson, 1962).

The peroxide value was calculated from the equation:

$$\text{Peroxide value (milliequivalent peroxide/kg sample)} = \frac{S \times N \times 1000}{\text{g sample}}$$

Where S = ml $Na_2S_2O_3$ (Test – Blank) and N = normality of $Na_2S_2O_3$

Determination of ester value

The ester value is a measure of the amount of ester present in the given oil sample and it is expressed in the same terms as saponification value and the acid value. It was calculated by subtracting the acid value from the saponification value (Andualem and Gessesse, 2014).

Ester value = Saponification value – Acid value

Statistical analysis

All the analyses were carried out in triplicate and analysed by Analysis of Variance (ANOVA) and the data were recorded as mean ± standard deviation (SD) (Raghava, 1987). The computer software employed for the statistical analysis was IBM SPSS Statistics, version 20 (USA).

RESULTS AND DISCUSSION

The physicochemical characteristics of *Garcinia gummi-gutta* seed oil were determined to understand the quality, purity and identification of the oil sample and these properties depends on the nature of the oil which were used for the characterization irrespective of sources of origin or location. The yield (%) of the oil extracted from the seeds was 15% and the physical state of the seed oil or seed butter is solid and it is quite hard at room temperature. The seed oil is yellowish brown in colour without any characteristic odour.

Moisture content of the sample indicates the amount of water content present in the oil sample and moisture has a great impact on the shelf life of products. The moisture content is directly reflected the conservation of the product in storage and for the product with lower moisture content has longer shelf-life. The high moisture content of the oil creates problem in transesterification and results in reduced shelf-life of the oil (Ibeto et al., 2012). The presence of moisture content in the oil sample also encourages hydrolysis and ease of oxygen attack (Manji et al., 2006). The moisture content (%) of *G. gummi-gutta* seed oil was determined as 9.27% (Table 1). The moisture content of the seed oil is in the range between 5.0% and 11.0%, the range of the moisture value of legumes (Aremu et al., 2006).

The melting point of the seed butter is high (39.8^{o}C) and it is helpful in preventing heat induced softening and loss of consistency of chocolates in hot climatic regions. Hence it can be used to increase the heat resistance effect and hardness of chocolates (Maheshwari and Reddy, 2005). The refractive index of oils depends on factors such as the length of fatty acid chain, molecular weight, degree of unsaturation and degree of conjugation. The range for refractive index of most of the oils is between 1.44 and 1.48 (Shahidi, 2005). The refractive index of *G. gummi-gutta* seed oil was determined as 1.460 (Table 1). It is in close agreement with castor oil (1.465), cashew nut oil (1.468), soya bean oil (1.470) and corn oil (1.470) (Akpan et al., 2007), which implied that *G. gummi-gutta* seed oil is less thicker and viscous than most of drying oils, where refractive indices are in the range from 1.475 to 1.485 (Duel and Tr, 1951).

G. gummi-gutta seed oil is insoluble in water but rapdly dissolved in non-polar solvents such as hexane and petroleum ether. The ash content of the food stuff indicates its inorganic constituents after the organic and volatile material have been oxidised completely during the process of incineration at 600^{o} C in a muffle furnace. It also represents the stability of products and the variation in ash content is due to the variation in inorganic compounds (Nwosu et al., 2014). The ash content of *G. gummi-gutta* seed oil was detrmined as 0.89±0.12 (Table 2). The low ash content of the seed oil showed that the oil is relatively stable to oxidative rancidity. The lower the ash value, the better the oil when considering stability (Achinewhu, 1990).

Along with the triglycerides, a small quantity of free fatty acids is usually present in oils and it is known as acid value or acid number. The acid value increases during storage and the keeping quality of the oil relies upon the free fatty acid content. It represents freshness and storage quality of the fats or oils. *G. gummi-gutta* seed oil contains 0.020 g oleic acid equivalents/g of oil sample and the acid value was determined as 3.07±0.18 mg KOH/g oil (Table 2). Acid number is defined as the mg KOH required to neutralize the free fatty acid content present in 1 g of the oil sample and the free fatty acid content is expressed as oleic acid equivalents. Acid value is the measure of susceptibility and the extent of decomposition and lesser the free fatty acid content, better the quality of fat or oil. The acid value of the oil sample (3.07 mg KOH/g oil) is lower when compared with sunflower oil (3.09 mg KOH/g oil) and olive oil (6.6 mg KOH/g oil) and higher than cashew nut oil (0.82 mg KOH/g oil) and castor oil (1.148 mg KOH/g oil) (Aremu et al., 2006; Akpan et al., 2007). The acid value of *G. gummi-gutta* seed oil is within the range of ASTM specification (0.4-4.0 mg KOH/g oil) (ASTM, 2002). The acid value of the oil is within the range of the acceptable limit for edible oils (≤ 10 mg KOH/g oil) (Balley, 1982).

Free fatty acid value is used as general indication of the condition and edibility of oils (Pearson, 1976). The lower levels of free fatty acids in oils indicates low levels of hydrolytic and lipolytic activities (Kyari, 2008). The acid value obtained for *G. gummi-gutta* seed oil (3.07 mg KOH/g oil) is lower when compared with that of coconut oil (5.5 mg KOH/g oil) and groudnut oil (9.0 mg KOH/g oil), indicates the lower level of the action towards which the glycerides had been decomposed by the action of lipase (Pearson, 1975).

Saponification can be defined as the process by which the fatty acids in the glycerides of the oil are hydrolyzed by an alkali. Saponification value is the amount of alkali required to saponify a definite quantity of fat or oil and this value is useful dor the comparison of the fatty acid chain length in oils (Horowitz, 1975). The saponification value of *G. gummi-gutta* seed oil was determined as 185.94±0.32 mg KOH/g oil. The saponification value of sunflower seed oil (197.43 mg KOH/g oil), palm kernel oil (280.5 mg KOH/g oil), groundnut oil (191.5 mg KOH/g oil and coconut oil (257.5 mg KOH/g oil) (Akinhanmi et al., 2008) are higher compared to *G. gummi-gutta* seed oil and which may indicate the presence of long chain fatty acids with high molecular weight in *G. gummi-gutta* seed oil. The oil sample with low sponification value contains relatively more of high molecular weight fatty acids and this is due to the presence of comparatively less number of carbohydrate functional groups per unit mass in long chain fatty acids. It is a rough index of the molecular weight of the oil or fat. According to Rossel (1984), the hugh saponification value indicate oxidation and its decrease indicate the unset of oxidation and it can be connected to the nature of oils (Magnus, 1992). The oils and fats with high saponification number yield quite soluble soaps and it also indicates the presence of high percentage of fatty acids in the oil sample (Omolara and Dosumu, 2009). The saponification value of *G. gummi-gutta* seed oil is within the same range of some edible oils reported by Eromosele and Paschal (2002) and ASTM (2002) specification.

Iodine value indicates the measure of the degree of unsaturation in an oil and it is constant for a particular fat or oil. It is a useful parameter to assess the oxidative rancidity of oils since higher the level of unsaturation the greater the possibility of the oils to go rancid. The principle of the test is that iodine gets incorporated into the fatty acd chain wherever the double bond exist. Hence the measure of iodine absorbed by an oil gives the degree of unsaturation. Iodine value is expressed as the grams of iodine absorbed by 100 grams of the oil (Horowitz, 1975). The iodine value of *G. gummi-gutta* seed oil was determined as 43.86 g/100 g oil and it is lower than sunflower oil (131.6 g/100 g oil), soyabean oil (109 g/100 g oil) and olive oil (94 g/100 g oil) and iodine value of an oil preferably should be in the range of 25-50 g/100 g oil and more preferably 30-45 g/100 g oil in good fat. The iodine value of coconut oil is very low (10.5 g/100 g oil) and hence it shows high tendency to go rancid. The high iodine value indicates dehydrogenation and decrease in iodine value suggests lipid oxidation and this may be due to the presence of metallic ions among other factors that promotes oxidation after the formation of hydroperoxide (Chan and Cotton, 1987). *G. gummi-gutta* seed oil can not be considered as drying oil since drying oils have an iodine value above 100 g/100 g oil (Duel and Tr, 1951).

Rancidity of the oil is caused by the action of air or by microorganisms giving the oil an unpleasant odour or flavour (McGinley, 1976). The rancidity caused by air is oxidative rancidity and ketonic rancidity is caused by microorganisms. In oxidative rancidity, oxygen is taken up by the fat with the formation of peroxides. The formed hydroperoxides subsequently decomposed to secondary oxidation products on storage, majority of which have unpleasant odour (Jambunathan and Reddy, 1991). The time taken for the development of rancidity and degree of peroxide formation differ among oils and fats. In the present study, peroxide formation is determined by titration against thiosulphate in the presence of potassium iodide (Rao and Deshpande, 2005). The high peroxide value indicates that the oil can easily go rancid, therefore, they are unstable and have short shelf life (Nzikou et al., 2007). The peroxide value of *G. gummi-gutta* seed oil was determined as 3.47±0.18 meq/kg and it is lower than that of olive oil (20 meq/kg) and sunflower oil (12.6 meq/kg) and higher than soyabean oil (0.32 meq/kg) (Campbell, 1983; Rehman et al., 2004). The peroxide values of the non edible oils were in the range of 4.36-9.82 meq/kg (Ibeto et al., 2012) which are higher than that of *G. gummi-gutta* seed oil (3.47 meq/kg). The ester value of the seed oil sample was determined as 182.87±0.55. The pH value of the oil sample was 4.69 and it is acidic.

Table 1. Physical properties of the oil sample extracted from *Garcinia gummi-gutta* seeds

Sl. No.	Parameters	Results
1	Physical state at room temperature	Solid
2	Colour	Yellowish brown
3	Odour	Odourless
4	Oil content (%)	15.10
5	Moisture content (%)	9.27
6	Refractive index	1.460
7	Melting point	39.8

Table 2. Chemical characteristics of the oil sample extracted from *Garcinia gummi-gutta* seeds

Sl. No.	Parameters	Results
1	Solubility	Soluble in non-polar solvents
2	pH	4.69
3	Ash content (%)	Not more than 0.89 ± 0.12
4	Free fatty acids (g oleic acid equivalents/g oil)	0.02 ± 0.10
5	Acid value (mg KOH/g oil)	3.07 ± 0.18
6	Saponification value (mg KOH/g oil)	185.94 ± 0.32
7	Iodine value (g/100 g oil)	43.86 ± 0.24
8	Peroxide value (meq/kg)	3.47 ± 0.18
9	Ester value	182.87 ± 0.55

CONCLUSION

The present study deals with the information regarding the extraction and evaluation of physicochemical characteristics of *Garcinia gummi-gutta* seed oil. The physicochemical characteristics such as acid value, saponification value and peroxide value of *G. gummi-gutta* seed oil are within the range of domestic oils used for cooking purposes and therefore it forms an important source for edible oil. The seed oil is solid and hard at room temperature due to the higher presence of saturated fatty acids and this property makes the seed butter convenient to use along with cocoa butter in confectionaries to prevent heat induced softening of chocolates. Further detailed studies on nutritional and anti-nutritional components of the seed oil is essential to confirm the edibility of the seed oil.

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