Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [8] July 2018 : 98-102

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Biochemical characterization of tea (*Camellia* spp) seed oil cake

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ABSTRACT
The biochemical characterizations of tea seed oil cake of eight commercial bi-clonal seed stocks were studied at two developmental stages. Standard chemical methods were followed for detection of phytochemicals, extraction and estimation of saponin, nitrogen, phosphorus, potassium and starch in the tea seed oil cakes. The saponin (%) , nitrogen (%), phosphorus (μg/g), potassium (μg/g) and starch (%) of the seed cake ranged between 2.41-8.08, 1.19-2.93, 2.42-3.68, 0.11-0.18, 27.29-70.77, respectively. The starch content estimated by fermentation process was found to be 18.35 to 50.39%. The present study indicated that the tea seed oil cake can be a potential source for extraction of starch and saponin. The seed cake may also have possible utility as organic manure as a source of macroelements like nitrogen and phosphorus.
Key words: Characterization, Bi-clonal, Saponin, Fermentation, Starch

Received 18.05.2018 Revised 23.06.2018 Accepted 01.07.2018

INTRODUCTION

Tea is one of the most popular beverages which is manufactured from the leaves or the young tender shoots of the tea plants, *Camellia sinensis* (L.) O. Kuntze. Since, tea has so far been used only as beverage, the leaves or the new vegetative growth is considered to have economic importance. India being the highest producer of tea, and famous for her characteristic types of tea, no thought has been given for alternative use of tea plant. So far, tea seeds are produced for production of planting material. Under normal growing condition, after planting, from third year onward seeds can be harvested. At this stage, the seed yield is maximum 6.42qtls / ha, under triangular planting system with 3m x 3m spacing. The yield doubles after 5 years of planting and it becomes about six times after 8 years of planting [4].

Tea seed oil is extracted on commercial scale in China. The residue (cake) left after extraction of oil, contains saponin, a tri-terpenoid glycoside which has multiple utilities such as detergent, pesticide, etc. There was 5-8% of tea saponin in *Camellia* seed shell, and the tea saponin could be extracted and be widely used in chemical, pharmaceutical, building materials, food, textile, printing, pesticides, aquaculture and other industries [21, 23]. It has been reported that saponin in tea seed could lower the serum levels of cholesterol, triglycerides, and low density-lipoprotein in rats [14,15]. Tea saponin, consisting of triterpenoid saponins, structural sugar and acid, was traditionally extracted from tea oil of C*amellia* seeds [24]. The pure tea saponin is milky white or pale yellow solid amorphous powder, and its melting point ranged from 223 to 224 ºC. The molecular formula and the relative molecular mass to be $C_{57}H_{90}O_{26}$ and 1203, respectively [16,22]. It is reported that the crude saponin content in the defatted seed meal of *C. oleifera* was 8.34% and the total saponin content in the crude saponin extract was 39.5% (w/w). The saponin content in the defatted seed meal of *C. oleifera* is higher than other traditional Chinese medicinal plants [3]. Tea saponin in seed shell of tea *Camellia* is about 5% to 8%, which increased substantially the secondary value of the tea oil of *Camellia* [12,25]. The nitrogen, phosphorus and potassium content in six tea clones were reported to be in the range of 1.9% to 2.7%, 1.1 μg/g to 3.8μg/g and 0.10 μg/g to 0.17μg/g, respectively. The macro-elements in the de-oiled tea seed oil cake in the order from the highest abundant mineral to the least abundant mineral to be N, Ca, Mg, P, Na and K, respectively [17]. Tea seeds contained about 18% starch and about 20% protein and the tea seed starch yield obtained by the fermentation process was 16%. They also reported that the starch extracted by fermentation process had no any disliked smells and were completely in conformity with government standards for edible starch [11].

Considering paucity of data on the above mentioned field for the seed stocks of cultivated germplasm of Assam, and also to find out the alternative use of tea, the present study was proposed with the following objective *i.e.* to evaluate the biochemical properties of tea seed oil cake.

MATERIALS AND METHODS

Sample collection

The tea fruit of the different clones were collected from the seed *bari* approved by Tocklai Tea Research Institute, Jorhat, Assam. The fruit of the different clones were collected at two developmental stages *viz.* seven and eight months (fully matured) after seed development. The clones collected for the present study were TS-378, TS-379, TS-462, TS-463, TS-464, TS-491, TS-506 and TS-520. The fruit were stored at -20ºC until these were used for further analysis. The cotyledons, manually separated from the seeds were used for various biochemical analysis.

Chemicals

Chemicals used in the present study were collected from Sisco Research Laboratories Private Limited, Andheri, Mumbai, Maharashtra. All the chemicals were of analytical grade.

Biochemical characterization of tea seed oil cake

The tea seed oil cake recovery (%, dry weight basis) of different bi-clonal seed stocks at two stages of seed development *viz.* seven and eight months after seed formation were determined by subtracting the crude oil percentage from 100 [19]. Standard chemical methods were followed for detection of phytochemicals [8], extraction and estimation of saponin [18], nitrogen [1], phosphorus [6], potassium [10] and starch [9] in the tea seed oil cakes.

Estimation of starch by fermentation process

The fermentation process [11] was followed to extract starch from the matured seeds (eight months after seed formation) from different tea seed stocks. Initially, in static fermentation the shell of tea seeds was separated with husking machine. Then endopleura of seeds were removed from seeds, the seeds without shell were soaked in water of 25 °C for 12-16 h. After that, the seeds without endopleura were grinded mixing with water (1.7/1 of water/seed; v/v) into thick liquid using mixer grinder. The thick liquid was then filtered with muslin cloth. Finally, the filtered liquid was poured into beaker and the beaker was placed in incubator at 45 °C, for 48 hrs. When fermentation went normally, the fermented liquid was divided naturally into three layers, milky white top layer, deep yellow middle layer and white bottom layer.

Separation of fermented liquid was done by collecting white top layer with a big spoon into another beaker. Then, the deep yellow middle layer was poured carefully and slowly. Finally, white bottom layer was kept in the primary beaker. The white bottom layer consisted of starch. To make the starch more pure, pure water at 45°C was poured into beaker (starch/water 1:5, v/v), stirred enough, and then allowed to deposit the starch. To clear away other soluble materials from starch, the washing process with normal water was repeated more than five times. Finished starch was collected after sun drying.

RESULTS AND DISCUSSION

Tea seed oil cake recovery

Tea seed oil cake recovery of different bi-clonal seed stocks at two stages of maturity (Table 1), showed that there was decrease in oil cake percentage as the oil content increased with seed maturity, being the highest and the lowest values were observed for bi-clonal seed stock TS-491 (88.98 to 89.24%) and TS-379 (73.16 to 76.69), respectively. However, it was earlier reported that the tea seed oil cake constituted about 65% of tea seeds [20].

Phytochemicals

The phytochemicals (Table 2) qualitatively detected in tea seed oil cake of different bi-clonal seed stocks were alkaloids, terpenoids and flavonoids, but the steroids were absent. However, none of the above phytochemicals was detected in starch extracted by fermentation process.

Biochemical quality analysis of tea seed oil cake

The biochemical quality of tea seed oil cake (saponin content, nitrogen, phosphorus, potassium and starch content) of eight bi-clonal seed stocks (Table 3) showed that the saponin content of different bi-clonal seed stocks ranged between 2.41 and 8.08 %. The saponin content was found to be the highest in TS-462 with 6.52% after seven months of seed formation and in TS-491 with 8.08 % after eight months of seed formation. It was found to be the lowest in TS-464 at both the stages, which ranged between 2.41% and 2.45%. Earlier it was reported that the saponin content in tea seed cake were 5 to 8%, 8%, 5% and 8%, respectively [3,12,21,23,25].

Table1. Tea seed oil cake recovery (%, dry basis) of different bi-clonal seed stock

Bi-clonal seed stock	Mean percentage (%) of seed cake	
	A	B
TS-378	81.30	80.04
TS-379	76.69	73.16
TS-462	82.86	78.61
TS-463	86.52	84.13
TS-464	79.89	79.38
TS-491	89.24	88.98
TS-506	81.94	81.03
TS-520	84.89	84.35

A, seven months after seed formation; B, eight months after seed formation

Table 2. Phytochemicals in tea seed oil cake and starch (fermentation process) at eight months after seed formation

Bi-clonal seed stock	Alkaloids		Terpenoids		Steroids		Flavonoids	
	TSOC	Starch(F)	TSOC	Starch(F)	TSOC	Starch(F)	TSOC	Starch(F)
TS - 378	+	-	+	-	-	-	+	-
TS - 379	+	-	+	-	-	-	+	-
TS - 462	+	-	+	-	-	-	+	-
TS - 463	+	-	+	-	-	-	+	-
TS - 464	+	-	+	-	-	-	+	-
TS - 491	+	-	+	-	-	-	+	-
TS - 506	+	-	+	-	-	-	+	-
TS - 520	+	-	+	-	-	-	+	-

TSOC, Tea seed oil cake; (F), Fermentation

Table 3. Biochemical quality of tea seed oil cake

Bi-clonal seed stock	Saponin content (%)		Nitrogen (%)		Phosphorus (µg/g)		Potassium (µg/g)		Starch content (g/100g)		
	A	B	A	B	A	B	A	B	A	B	B*
TS - 378	4.26	4.37	2.56	2.63	4.08	3.50	0.17	0.17	44.47	48.15	31.56
TS - 379	5.21	5.32	1.80	1.84	3.02	3.10	0.17	0.16	27.29	25.69	18.35
TS - 462	6.52	6.47	1.61	1.66	2.64	3.46	0.18	0.18	60.99	60.86	41.62
TS - 463	3.72	3.96	1.63	1.46	2.76	2.54	0.11	0.11	54.15	53.26	40.81
TS - 464	2.41	2.45	1.19	1.54	2.84	2.92	0.14	0.13	56.55	55.05	40.82
TS - 491	5.35	8.08	2.22	2.93	3.38	3.26	0.12	0.12	68.87	66.52	50.39
TS - 506	2.65	2.84	1.67	1.66	2.42	2.51	0.11	0.11	56.02	56.47	39.14
TS - 520	3.52	3.29	2.17	2.26	3.67	3.68	0.12	0.12	69.28	70.77	49.23
Mean	4.20	4.59	1.85	2.00	3.10	3.12	0.14	0.14	54.70	54.59	38.99
S.Ed(±)	0.07	0.08	0.018	0.016	0.35	0.59	0.001	0.007	1.93	3.11	0.53
$CD_{t\ 0.05}$	0.13	0.15	0.031	0.028	0.62	1.04	0.003	0.013	3.36	5.43	0.93

A: seven months after seed formation, B: eight months after seed formation
B*: extracted by fermentation process

The nitrogen percentage of different bi-clonal seed stocks ranged between 1.19% and 2.93 %. The nitrogen percentage was found to be the highest in TS-378 with 2.56 % after seven months of seed formation and in TS-491 with 2.93 % after eight months of seed formation. It was found to be the lowest in TS-464 with 1.19% after seven months of seed formation and in TS-463 with 1.46% after eight months of seed formation. There are reports of nitrogen content of *Assam* variety, Cambod variety and *China* variety clones to be 1.9% to 2.7% [17]. The nitrogen content of maize germ meal, sunflower meal and soybean meal were reported to be 2.8%, 4.9% and 8.3%, respectively [17].

The phosphorus content of different bi-clonal seed stocks ranged between 2.42 and 4.08 μg/g. After seven months of seed formation, the highest phosphorus content of 4.08 μg/g was found in TS-378 and the lowest (2.42 μg/g) was found in TS-506. After eight months of seed formation, the highest phosphorus content of 3.68 μg/g was found in TS-520 and the lowest (2.51 μg/g) was found in TS-506. The phosphorus content of Assam variety, Cambod variety and *China* variety clones were in the range between 1.1 μg/g to 3.8μg/g [17]. The phosphorus content of maize germ meal, sunflower meal and soybean meal were reported to be 7.2 μg/g, 6.0 μg/g and 5.5 μg/g, respectively [17], which were higher compared to phosphorus content of tea seed oil cake observed in the present study. There is a higher level of phosphorus in sesame oil cake (0.69% to 1.11%) and de-oiled canola oil cake (1.06%) [5,13]. The phosphorus content of mustard, sunflower and soybean oil cake was reported to be 700 mg /100g, 670 mg/100g and 690mg /100g, respectively [7].

Potassium content was found to be the highest in TS-462 (0.18 μg/g) and the lowest (0.11 μg/g) in both TS-463 and TS-506. Earlier, it was reported that the potassium content of *Assam* variety, Cambod variety and *China* variety clones was in the range between 0.10 μg/g to 0.17μg/g [17].

The starch content of different bi-clonal seed stocks ranged between 25.69 g/100g and 70.77g/100g. The starch content in both the stages were found to be the highest (69.28 and 70.77 g/100g) in TS-520. It was found to be the lowest (25.69 and 27.29 g/100g) in TS-379. There are reports on the starch content of tea seed meal on dry weight basis to be 30 to 50% [2]. The starch content in mustard, sunflower and soybean oil cake were found to be 23.8 g/100g, 17.9 g/100g and 20.9 g/100g, respectively [7], which were much lower compared to starch content of tea seeds observed in the present study.

Starch extracted by fermentation process

The starch (Table 3) extracted by fermentation process was found to be 18.35 (TS-379) to 50.39 % (TS-491) which was lower as compared to starch (25.69 to 70.77 %) estimated in tea seed oil cake by chemical extraction. However, there was report of lower starch yield extracted by fermentation process (16 %) [11].

CONCLUSIONS

The present study indicated that the tea seed oil cake can be a potential source of starch, saponin and other macroelements like nitrogen and phosphorus. The fermentation process can be successfully utilized for extraction of starch from tea seed oil cake on commercial basis. The bi-clonal seed stocks TS-491 and TS-520 were found to be prominent from biochemical characterization. The seed cake may also have possible utility as organic manure as a source of macroelements like nitrogen and phosphorus. The present study revealed the possibility of alternative use of tea particularly for tea growing regions.

AKNOWLEDGEMENTS

We thank the Department of Science and Technology, India, for offering INSPIRE FELLOWSHIP to the first author.

REFERENCES

1. A.O.A.C.(1965). 10th Edition, Washington DC.
2. Anonymous .(2009). The China State Forestry Bureau. The camellia industry development planning.
3. Chen, Y.F., Yang, C.H., Chang, M.S., Ciou, Y.P. and Huang, Y.C. (2010). Foam properties and detergent abilities of the saponins from *Camellia oleifera*. Int. J. Mol. Sci., 11: 4417–4425.
4. Das, S.C. and Konowor, B.K. (2002). Tea Seed Production. In: Notes on field management. Tea Research Association, Tocklai Experimentation Station, Jorhat.,pp 19-26.
5. Ewing, W. N. (1997).The Feeds Directory. Context Publications, Leicestershire, England.
6. Fiske, C.H. and Row, Y.S. (1925). The colorimetric determination of phosphorus. J. Biol. Chem.,66: 375.
7. Gopalan, C., Sastri, B.V.R. and Balasubramanium, S.C. (1971). Proximate Principles: Common Foods. In : Nutritive value of Indian Foods. Rao, B.S.N.; Deosthale, Y.G. and Pant, K.C. (Eds.) Offset press, ICMR, Hyderabad.
8. Harborne J.B. (1973). Phytochemical Methods, Chapman and Hall, Ltd., London, pp 49-188.
9. Hodge, J.E. and Hofreiter, B.T. (1962). In: Methods in Carbohydrate Chemistry (Eds. Whistler, R.L and BeMiller, J.N.), Academic Press, New York.
10. Jackson, M.L. (1962). Significance of kaolinite intersalation in clay mineral analysis. Clays clay min., 4:429.

11. Jiang, J., Ren, C., Wang, Z., Qian, C., Shen, Y. and Yang, Y. (2013). New Process for Extracting Oil and Starch from Tea Seeds . J. of Fd. Sci. Engg., 3:699-703.
12. Jie, L.H., Shao, P., Dan, F.H., Chang, L. and Ming, Y.X. (2012). Extraction of saponin from *camellia oleifera* cake and evaluation of its antioxidant activity. Intl. J. of Fd. Sci. and Technol., 47:1676-1687.
13. Kuo, L. H.(1967). Animal feeding stuffs compositional data of feeds and concentrates (Part 3). Malaysian Agric. J., 46: 63-70.
14. Lee, C.P. and Yen, G.C. (2006). Antioxidant activity and bioactive compounds of tea seed (*Camellia oleifera Abel.*) oil. J. Agric. Food Chem., 54:779-784.
15. Lee, C.P., Shih, P.H., Hsu, C.L. and Yen, G.C. (2007). Hepatoprotection of tea seed oil (*Camellia oleifera Abel.*) against CC_{l4}-induced oxidative damage in rats. Food Chem. Toxicol., 45: 888-895.
16. Lu, W.and Xiaoping, X. (1990). Evaluating the hydrophilic balance (HLB) values of tea saponin from its chemical structure. Nat. Prod. Res. and Dev., 2:33-37.
17. Njuguna, D. G., Wanyoko, J. K., Kinyanjui, T. and Wachira, F.N. (2013). Mineral elements in the kenyan tea seed oil cake. Intl. J. of Res. Chem. and Environ., 3: 253-261.
18. Obadoni, B.O. and Ochuko, P.O. (2001).Phytochemical Studies and Comparative Efficacy of the crude extracts of Some Homeostatic plant in Edo and Delta State of Nigeria, Global J. Pure Appl. Sci., 8: 203-208.
19. Sarmah, K., Das, P., Saikia, G.K. and Sarmah, T.C. (2016). Biochemical characterization of tea (*Camellia* spp) seed oil. In: International conference on neutraceuticals and functional foods-the challenges and oppurtunities, Department of Biochemistry, B.A. College of Agriculture, Anand Agricultural University, Anand.
20. Shi, W. Y. (2001). The comprehensive utilization of agricultural and sideline products. Zhejiang university publishing office, China.
21. Shujin, G., Yangshu, X. and Yuanjing, F. (1996). Studies on the techniques of processing the oil-tea camellia seed oil. J. of Anhui Agric. Sci., 24: 285-288.
22. Shuying, Y. (1998).Hunan Chem. Indus., 28: 31-32.
23. Xiansheng, L. (2005). Study on the development and utilization of Oil-tea Camellia. J. of Hunan Univ. of Sci. Engg., 26: 127-129.
24. Xiaojian, Q. and Ruifu, P. J. (1989). Chem. W. , 6: 265-277.
25. Yan, Y., Wu, Z.L., Zhao, Y.L. and Jiang, C.S. (2011). Separation of tea saponin by two-stage foam fractionation. Separation and Purification Technol., 2:12-13.

CITATION OF THIS ARTICLE

K Sarmah, P Das, G K Saikia and T C Sarmah. Biochemical characterization of tea (*Camellia* spp) seed oil cake. Bull. Env. Pharmacol. Life Sci., Vol 7 [8] July 2018 : 98-102