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RESEARCH ARTICLE

STUDIES ON THE SPENT WASTE OF (i) COFFEE AND (ii) TEA FOR EXTRACTION OF OIL, BIOACTIVE COMPOUNDS AND BIOETHANOL PRODUCTION

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ABSTRACT

Recent decades have seen a significant rise in coffee and tea production, consumption; consequently increase in their waste generation. Alternative routes are necessary for the waste management, to develop a new treatment that would be viable both technically and economically. The purpose of this study is to extract oil from the Spent Coffee Waste (SCW) and Spent Tea Waste (STW), individually with further extraction of bioactive compounds from coffee oil, as well as the production of bioethanol from the left-over solid waste after oil extraction. The extraction of oil was done using Soxhlet apparatus using hexane as solvent while bioactive compounds were extracted using hot saponification. After oil extraction, the residual waste was optimized for glucose level using acid hydrolysis and steam distillation for bioethanol production by fermentation using *Saccharomyces cereviciae*. Acid Hydrolysis provided the best bioethanol production from the SCW (5.78 g/L, 79.8 % efficiency) and Steam Distillation provided the best bioethanol production from STW (8.47 g/L, 79.4 % Efficiency). Coffee oil has High (86.92 %) antioxidant capacity comparing to Tea oil (52.57 %). The results showed the spent wastes of coffee and tea as potential feed stocks for the extraction of oil, bioactive compounds and bioethanol production.

INTRODUCTION

Recent decades have seen a significant rise in coffee and tea production, consumption; consequently an increase in their waste generation. India's annual coffee production is 58,500 Metric tons (Coffee Board of India, 2013-2014) and annual tea production is 1200,000 Metric tons (Tea board of India, 2013-2014). Although these wastes are generated in huge quantities every year, only a few studies have been made to address their reuse. Biomass is a potential renewable energy source that could replace fossil energy for transportation. The use of food crops (like corn, maize, sorghum) for biofuel production may cause inflation of cost of these crops leading to food insecurity. To alleviate such problems and based on the facts that large amount of coffee and tea waste generated every year, alternative and non-edible agricultural products like Spent Coffee Waste (SCW) and Spent Tea Waste (STW) are studied from both economical and environmental view points (Solange *et al.,* 2012).

Coffee

Coffee is a brewed beverage prepared from the roasted beans of several species of an evergreen shrub of the genus *Coffea*.

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Being the most popular drink in the world, the effect of coffee on human health has been a subject of many studies. The majority of recent research suggests that moderate coffee consumption is benign or mildly beneficial in healthy adults. India ranks 6th in the world for Coffee production with the annual average production of 6,58,500 Metric Tons and export of 3,47,855 Metric Tons (Coffee Board of India, 2013-2014)

Types of Coffee

The two most common sources of coffee beans are the highly regarded *Coffea arabica* and *Coffea robusta.* Coffee is slightly acidic (pH 5.0–5.1) and have a stimulating effect on humans because of its caffeine content.

Coffea arabica

C. arabica contains less caffeine and said to produce better tasting coffee than the other major commercially grown coffee species.

Coffea robusta

Roasted robusta beans produce a strong, full-bodied drink with a distinctive earthy flavour, but usually with more bitterness than *arabica* due to its pyrazine content.

Fig 1.1.2.1 Seeds of *Coffea Arabica*

Fig 1.1.2.2 Seeds of *Coffea arabica* and *Coffea robusta*

Coffee oil

The oil extracted from coffee is called as coffee oil. The extraction is performed with Soxhlet apparatus, using hexane as solvent in the laboratory (Julio *et al.,* 2006). Green coffee oil has been used in the cosmetics industry because of the emollient property provided by its fatty acids and its capacity to block sunlight harmful to human skin (Grollier *et al.,* 1988; Alvarez *et al.,* 2000). Oil from roasted coffee has also been widely used as a flavor source in food and cosmetics. Moreover, a reduction in the diterpene level of oil from roasted coffee significantly increases its stability and sensorial profile, decreasing its hypercholesterolemic effect (Bak *et al.,* 1989; Kolling *et al.,* 1999).

Table: Coffee oil

Description	Coffee plants naturally grow in South American rainforests. They produce red fruits which are roasted to produce coffee
Characteristics	The oil is thick and dark with a warming scent of freshly brewed coffee
Properties	Antioxidant, diuretic, stimulant, anticancer activity
Constituents	Caffeine, Diterpenes (Cafestol and Kahweol)
Uses	Coffee oil is used for flavoring confectionery such as chocolate and baked goods, cosmetic products such as sun blocks, deodorizer, skin care, body care and medical treatments for headaches, asthma and increasing blood pressure and heart and lung activity
Blends well with	Coffee oil is best used on its own but can be combined with peppermint, spearmint, or vanilla for a better sweet scent

Tea is an aromatic beverage commonly prepared by pouring hot or boiling water over cured leaves of the tea, *Camellia sinensis*.

After water, tea is the most widely consumed beverage in the world. It has a cooling, slightly bitter, and astringent flavor that many people enjoy. India ranks 2nd in the world for Tea production with annual average production of 12,00,000 Metric Tons and export of 1,47,000 Metric Tons (Tea Board of India, 2013-2014). Tea has long been promoted for giving a variety of positive health benefits. Green tea is also said to have anti-fibrotic properties and neuroprotective power. Tea catechins have known anti-inflammatory and neuroprotective properties.

Types of Tea

There are different types of tea consumed worldwide — Green tea, Black tea, Oolong tea and premium tea.

Tea Oil

The oil extracted from tea leaf is called as Tea Oil. The extraction is performed with Soxhlet apparatus, using hexane as solvent (Julio *et al.,* 2006). This is a sweetish seasoning and cooking oil.

Diterpenes from Coffee oil

Cafestol and Kahweol are the two naturally occurring diterpenes found only in coffee, are present in unsaponificable lipid fraction (Kolling *et al.,* 1999). Boiled coffee contains high concentration of these diterpenes resulting in hypercholesteromic effect, while the instant, drip-filtered coffee contains only negligible amounts (Bak *et al.,* 1989; Kolling *et al.,* 1999). The extraction of oil from SCW contains high concentration of diterpenes which are further extracted by hot saponification gives the positive effect such as antioxidant, anti-inflammatory and hepatoprotective (against cancer) activities (Dias *et al.,* 2013).

Cellulose

Carbohydrates are the important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The chemical properties of saccharides vary depending upon the number of hydroxyl groups and the presence or absence of –CHO/= CO groups. Cellulose, a major structural polysaccharide in plants, is the most abundant organic compound in nature, and is composed of glucose units joined together in the form of the repeating units of the disaccharide cellobiose with numerous cross linkages. It is also a major component in many of the farm wastes (Sadasivam, S. and Manickam. A., 1992).

Glucose

Glucose is a widely distributed simple sugar with an active aldehyde group which is considered of much importance for bioethanol production. Estimation of glucose by glucose oxidase gives the true glucose concentration eliminating the interference by other reducing sugars (Sadasivam and Manickam, 1992).

Ethanol

Ethanol also called ethyl alcohol is a volatile, flammable, colorless liquid with the structural formula CH_3CH_2OH. Best

known as the type of alcohol found in alcoholic beverages, it is also used in thermometers, as a solvent, and as a fuel. Ethanol for use in alcoholic beverages, and the vast majority of ethanol for use as fuel, is produced by fermentation and using agro-industrial waste as substrates is very attractive for its abundance, non-competition with foodstuffs and environmental aspects. When certain species of yeast (e.g., *Saccharomyces cerevisiae*) metabolize sugar in reduced-oxygen conditions they produce ethanol and carbon dioxide. *S.cereviciae* is the yeast ferments only hexoses is most used for ethanol production due to its ability to grow in media containing high sugar concentration and high ethanol yield. (Solange *et al.,* 2012). The chemical equations below summarize the conversion:

$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2$

Objectives

The aim of this study is

- To extract oil from the Spent Coffee Waste (SCW) and Spent Tea Waste (STW)
- To estimate the antioxidant capacity of Coffee oil and tea oil
- To extract bioactive compounds (diterpenes) from the coffee oil and test its anticancer activity and
- To produce Bioethanol from the left- over SCW and STW after oil extraction in the laboratory

MATERIALS AND METHODS

Collection of Sample

After brewing, the SCW and STW were collected from hotels and kept for drying under Sun. These dried spent wastes were subjected for the experimental analysis.

Extraction of Oil from SCW and STW Soxhlet Method

A method published by Julio *et al*., (2006) was adopted for oil extraction from SCW and STW.

Reagents required

n-Hexane

Protocol

- Weighed 20g of the sample into the thimble
- 150mL of hexane was taken into the round bottom flask
- The boiling point was set at 68-69ºC for boiling of hexane to evaporate and condense onto the sample in the thimble
- Warm hexane separates the oil from the sample and drips again into the round bottom flask. This referred to one cycle
- Repeated the process up to 8 cycles for 1 hour
- Finally round bottom flask contained both hexane and oil
- Now, the mixture was taken to flash evaporator for separation of hexane and oil
- Using flash evaporator, coffee oil was separated from hexane.

Determination of Total Phenolic content

A method provided by Singleton *et al.,* (1965) was adopted for determining total phenolics in the coffee oil and tea oil.

Reagents required

- 7.5 %(w/v) Sodium carbonate
- Folin-Ciocalteau Reagent
- Tannic acid

Protocol

- 5 µL of filtered extract was mixed with 60 µL of sodium carbonate and 15 µL of Folin-Ciocalteau reagent
- Subsequently, 200 µL of distilled water were added and the solutions were mixed.
- After that, the samples were heated at 60ºC for 5 min and were allowed to cool to room temperature
- The absorbance was measured by means of spectrophotometer at 700nm
- A calibration curve was made from a Tannic acid standard solution (200, 400, 00, 800, 1000, 2000, 3000 mg/L) and the blank was prepared with distilled water
- The total content of phenolic compounds was expressed as milligram tannic acid equivalent per dry weight material (mg TAE/g SCW) and (mg TAE/g STW).

Determination of Antioxidants by DPPH assay

The scavenging activity of natural antioxidants in coffee oil and tea oil towards the stable free radical DPPH was measured by the method of Mensor *et al.,* (2001)

Reagents required

- DPPH (2,2-Diphenyl 2- Picryl hydrozyl Hydrate)-0.3mM in methanol
- Methanol

Protocol

- Leaf extracts (20µL) were added to 0.5mL of methanolic solution of DPPH and 0.48mL of methanol.
- The mixture was allowed to react at room temperature for 30 min.
- Methanol served as blank and DPPH in methanol, without leaf extracts served as positive control.
- After 30 min of incubation, discolouration of purple colour was measured at 518nm.
- The radical scavenging activity was calculated as follows:

Scavenging activity (%) = (C-T)/C * 100

Extraction of Diterpenes from Coffee oil

Hot Saponification

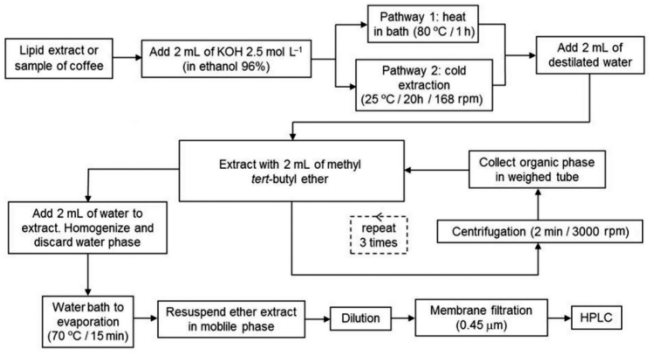

Fig 3.5. Flowchart of Diterpene extraction from roasted coffee

The method described by Dias *et al*., (2013) was employed for diterpene extraction.

Reagents required

- 2.5 M KOH
- n-Hexane

Protocol

- 50 µL of the coffee oil was taken and then 2 mL of KOH solution was added for direct sample saponification.
- After 1 h boiling for 80 ºC, the unsaponifiable matter was extracted using hexane and washed with water (pathway 1, Figure 3.5).

Determination of Cellulose Content

The identification of cellulose was made following the method described by Sadasivam, S and Manickam, A (1992)

Reagents required

- ***Acetic/Nitric reagent:*** Mixed 150 mL of 80% acetic acid and 15 mL of concentrated nitric acid.
- ***Anthrone reagent:*** Dissolved 200 mg anthrone in 100 mL concentrated sulphuric acid. Prepared fresh and chill for 2 h before use.
- 67% sulphuric acid.

Protocol

- Added 3 mL acetic/nitric reagent to a known amount (0.5 g or 1 g) of the sample in a test tube and mixed in a vortex mixer
- Placed the tube in a water-bath at 100°C for 30 min
- Cooled and then centrifuged the contents for 15–20 min
- Discarded the supernatant
- The residue was washed with distilled water
- Added 10 mL of 67% sulphuric acid and allowed it to stand for 1 h
- Diluted 1 mL of the above solution to 100 mL
- To 1 mL of this diluted solution, 10 mL of anthrone reagent was added and mixed
- Heated the tubes in a boiling water-bath for 10 min
- Cooled and measured the colour at 630 nm
- Set a blank with anthrone reagent and distilled water
- Taken 100 mg cellulose in a test tube and proceeded from Step No. 6 for standard

Instead of just taking 1 mL of the diluted solution (Step 7) taken a series of volumes. (say 0.4–2 mL corresponding to 40–200 µg of cellulose) and developed the colour

IDENTIFICATION OF GLUCOSE

A method provided by Sadasivam,S. and Manickam,A., (1992) was adopted for identification of glucose.

Reagents required

Benedict's qualitative reagent: Dissolved 173 g sodium citrate and 100 g sodium carbonate in about 500 mL water. Heated to dissolve the salts and filtered, if necessary. Dissolved 17.3 g copper sulphate in about 100 mL water and added it to the above solution with stirring and made up to the volume to 1 L with water.

Protocol

Benedict's Test To 2mL of Benedict's reagent added five drops of the test solution. Boiled for five minutes in a water bath. Cool the solution and colour change was visualized.

Determination of glucose content by glucose oxidase method

A method provided by Sadasivam,S. and Manickam,A., (1992) was adopted for identification of glucose.

Reagents required

Glucose Oxidase Peroxidase Reagent

Dissolved 25 mg O-dianisidine completely in 1 mL of methanol. Added 49 mL of 0.1 M. phosphate buffer (pH 6.5). Then added 5 mg of peroxidase and 5 mg of glucose oxidase to the above prepared O-dianisidine solution.

Standard: Dissolved 100 mg glucose in 100 mL water. Diluted 10 mL of this stock to 100 mL to obtain the working standard.

Protocol

- To 0.5 mL of deprotinised plant extract (deproteinization is not necessary in samples with very low protein content) 0.5 mL distilled water and 1 mL glucose oxidaseperoxidase reagent added
- Into a series of test tubes pipetted out 0 (blank), 0.2, 0.4, 0.6, 0.8 and 1 mL of working standard glucose solution and the volume was made up to 1.0 mL with distilled water
- Then added 1 mL of glucose oxidase-peroxidase reagent
- All the tubes were incubated at 35°C for 40 minutes
- Terminated the reaction by the addition of 2 mL of 6 N-HCl
- Read the colour intensity at 540 nm

PRODUCTION OF BIOETHANOL

ACID HYDROLYSIS

- Weighed 5g of the sample into 50 ml of H2SO4 ranging with different concentration (1 %, 2%, 3%, 4% and 5%) in the solid to liquid ratio of 1:10 for optimizing the acid concentration
- Refluxed the mixture for different time periods 15, 30, 45 and 60 minutes for the optimization of glucose content in the hydrolyzed sample
- This hydrolyzed sample was supplied as the carbon source for bioethanol production

STEAM DISTILLATION

- Weighed 5g of the sample into 50 ml of distilled water in the solid to liquid ratio of 1:10
- Refluxed the mixture for different time periods of 1, 2, 3 and 4 hours for the optimization of glucose content in the refluxed sample
- This steam distilled sample was taken as the carbon source for bioethanol production

Inocula And Fermentation Conditions

Culture medium described by Solange *et al.,* (2012) was followed for fermentation using *S.cereviciae*

Culture Medium (g/L)

Glucose (1%, 2%, 3%, 4%, 5% of hydrolyzed sample)
$(NH_4)_2H_2PO_4$ - 3g/L
$MgSO_4.7H_2O$ - 1g/L
Yeast extract - 3g/L

Protocol

- For the inoculum preparation, 3 g/L of S.cereviciae (Bakers yeast) was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of culture medium, pH 5.5
- Concentrated solutions of each compound were prepared individualy and sterilized in an autoclave at 121 ºC for 20 min
- The inoculated flasks were incubated on a rotary shaker at 30 ºC, 100 rpm, for 12, 24, 36 and 48 h period. After this time, the cells were recovered by centrifugation at 5000 rpm for 20 min
- The supernatant was taken to flash evaporator for the separation of produced bioethanol from the medium
- Then, acidified dichromate assay was performed for determining the bioethanol concentration (as described below)
- The ethanol yield factor (YP/S, g/g) was defined as the ratio between the maximum ethanol concentration (g/l) and total glucose consumed (g/l)
- The efficiency of glucose conversion to ethanol (η, %) was determined as the ratio between the obtained YP/S (g/g) and the theoretical value (0.51 g/g) of this parameter

Estimation of bioethanol by acidified dichromate assay

The method published by Caputi *et al.,* (1968) was adopted for the estimation of bioethanol.

Reagents required

Acidified dichromate reagent Dissolved 34 g of potassium dichromate in 325mL of concentrated H_2SO_4, and made up to 1 liter.

Working standard 1mL of ethanol in 100mL of distilled water.

Protocol

- A calibration curve was constructed by using ethanol standard solution (1, 2, 3, 4 and 5 mL) by adding to 10mL of the reagent
- Blank was set with distilled water and 10mL of the reagent
- To 2mL of the test sample, 10mL of the reagent was added
- The samples were heated at 60ºC for 20 min and cooled to room temeperature
- The absorbance of green colour was measured in a spectrophotometer at 600nm

RESULTS AND DISCUSSION

Extraction and separation of oil from spent coffee waste (scw)

Fig 3.1 Coffee Oil from SCW

Each 100 g of Spent Coffee Waste yielded 12.5 mL of oil.

Extraction and separation of oil from spent tea waste (stw)

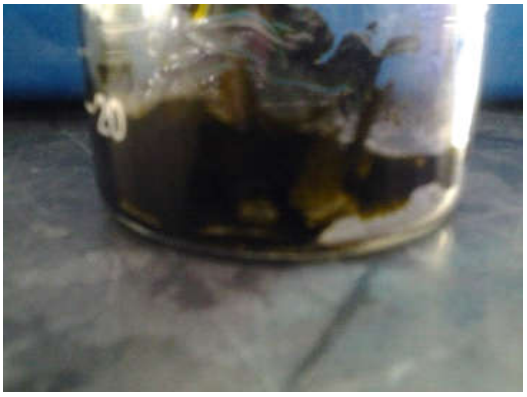

Fig 3.1 Tea Oil from STW

Each 100g of Spent Tea Waste yielded 2.5mL of oil.

Table 3.1. Extraction of oil from SCW

Sample	Volume of the sample (g)	Volume of hexane (mL)	No. of cycles	Total time taken (h)	Volume of the oil obtained (mL)	Volume of hexane recovered (%)
Spent coffee waste	10	75	8	1	1.25	63

Table 3.1 Extraction of oil from STW

Sample	Volume of the sample (g)	Volume of hexane (mL)	No.of cycles	Total time taken (h)	Volume of the oil obtained (mL)	Volume of hexane recovered (%)
Spent Tea waste	10	75	16	2	0.25	63

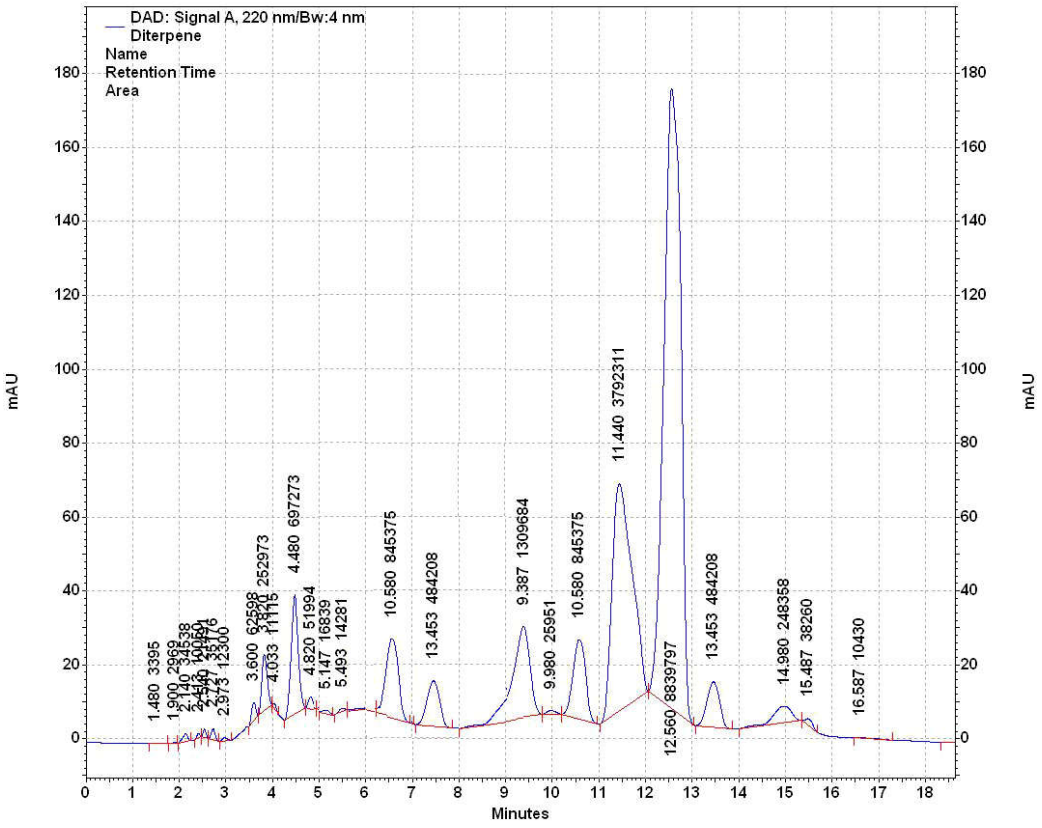

Fig 4.5. Qualitative analysis of diterpenes in coffee oil by HPLC-DAD

Determination of total phenolic content

Table 4.3. Total Phenolics content

SAMPLE	TOTAL PHENOLICS (mg TAE/mL)
Coffee oil	78.4
Tea oil	62.8
Hydrolyzed Coffee	25.2
Hydrolyzed Tea	20.6

Determination Of Antioxidants By Dpph Method (I) Coffee Oil And (Ii) Tea Oil

Table 4.4 Antioxidant capacity by DPPH method

SAMPLE	SCAVENGING ACTIVITY (%)
Coffee oil	86.92
Tea oil	52.57

Qualitative Analysis Of Diterpenes In Coffee Oil By Hplc-Dad

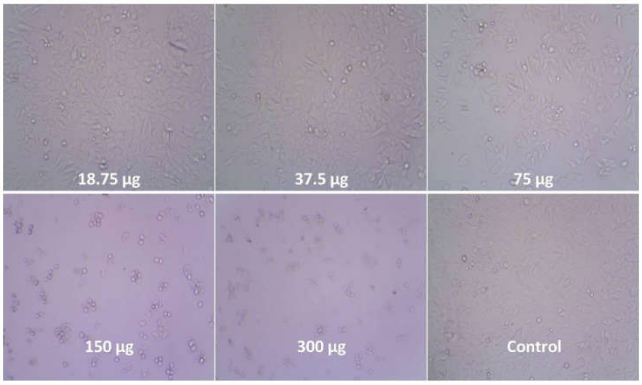

Fig 4.6 Anticancer activity of Cancer Cell Lines

300µg of Diterpenes has good anticancer activity for Breast Cancer.

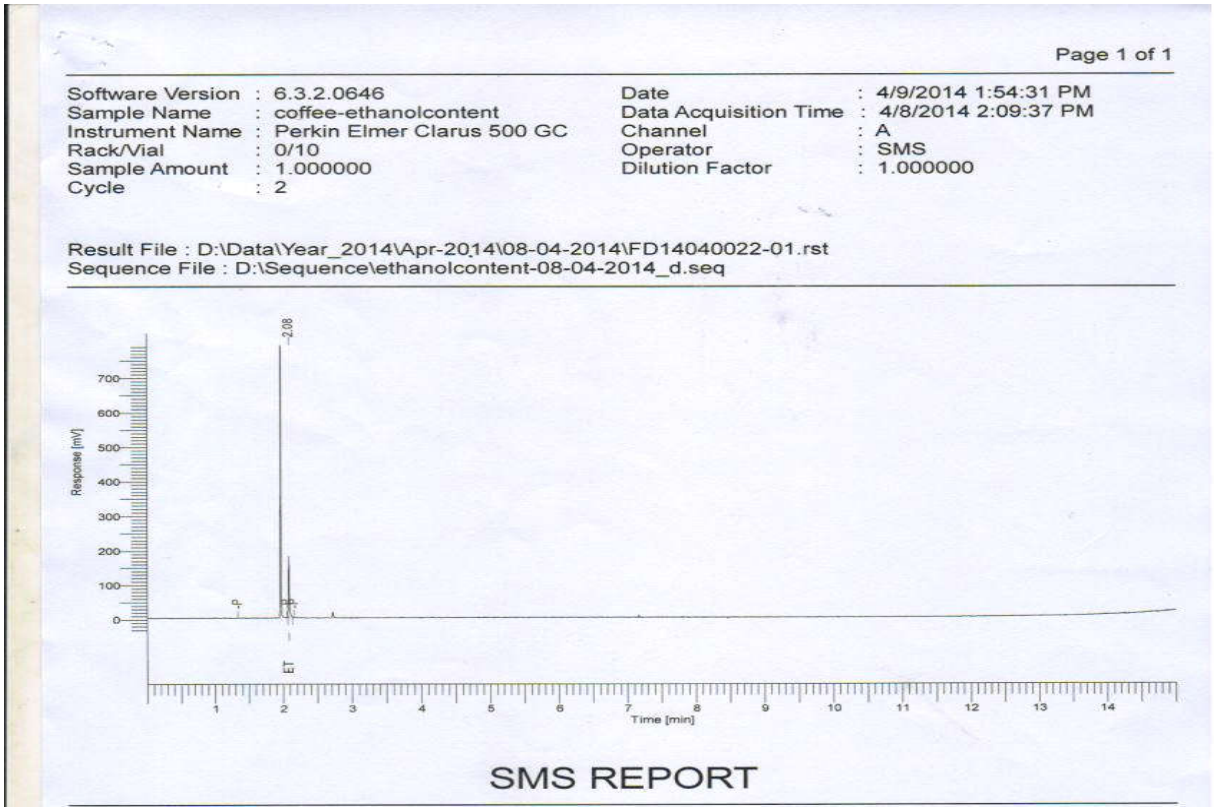

Fig 4.11.1 Quantitative analysis of bioethanol by GC-MS for SCW

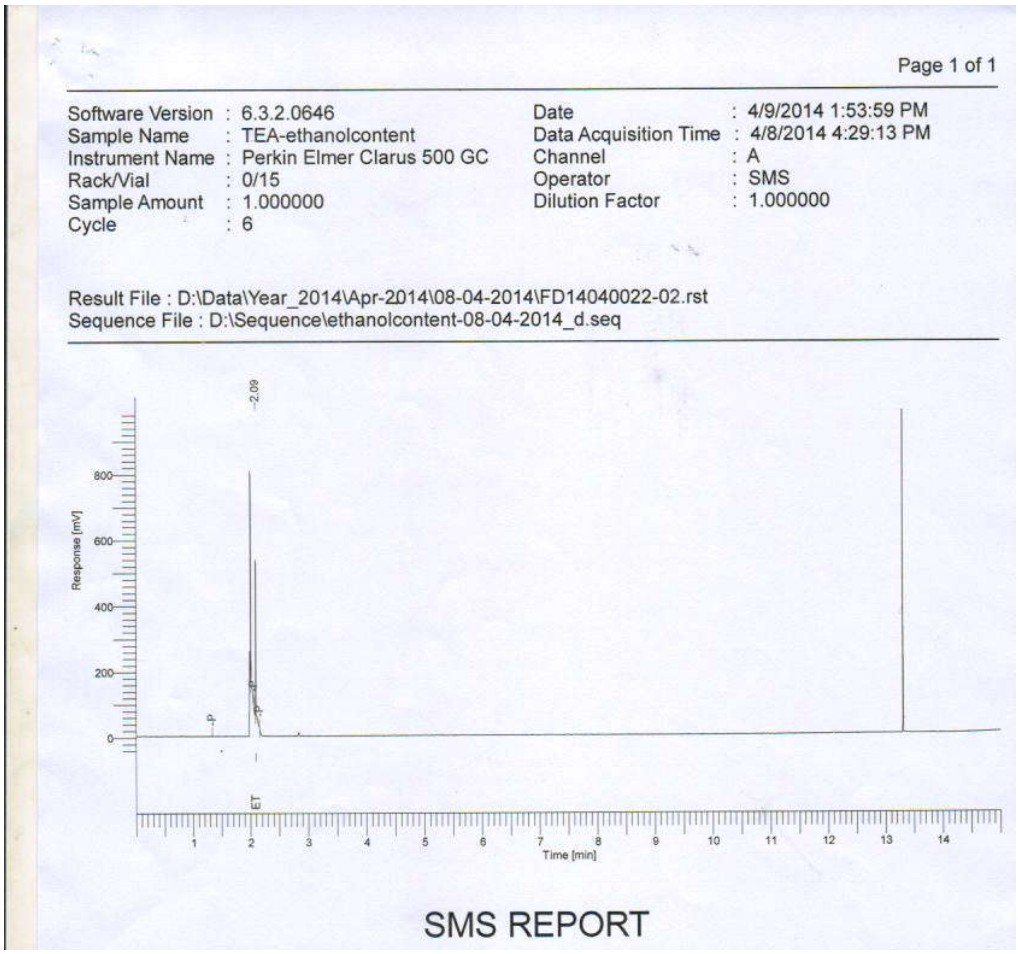

Fig 4.11.1 Quantitative analysis of bioethanol by GC-MS for STW

Determination of Cellulose In Scw And Stw

Table 4.7. Cellulose Content in SCW and STW

MATERIAL	CELLULOSE %
SCW-Before oil extraction	27.75
SCW-After oil extraction	22.75
STW- Before oil extraction	25.85
STW-After oil extraction	20.65

Optimiation of glucose content by acid hydrolysis in scw and stw

Table 4.8.1 Optimiation of glucose content by acid hydrolysis in SCW

Time Vs Acid Concentration (Sulfuric Acid)	15 min	30 min	45 min	60 min
	GLUCOSE CONTENT (mg/mL)			
1 %	0.194	0.329	0.174	0.147
2 %	0.139	0.174	0.119	0.094
3 %	0.123	0.158	0.103	0.074
4 %	0.106	0.14	0.088	0.062
5 %	0.091	0.124	0.074	0.043

Table 4.8.2. Optimiation of glucose content by acid hydrolysis in STW

Time vs acid concentration (sulfuric acid)	15 min	30 min	45 min	60 min
	GLUCOSE CONTENT (mg/mL)			
1 %	0.214	0.277	0.210	0.192
2 %	0.183	0.247	0.174	0.16
3 %	0.163	0.22	0.147	0.135
4 %	0.138	0.196	0.12	0.113
5 %	0.126	0.188	0.106	0.108

Optimization Of Glucose Content By Steam Distillation From Scw And Stw

Table 4.9.1 Optimiation of glucose content by Steam distillation in SCW

TIME (h)	GLUCOSE LEVEL (mg/mL)
1	0.107
2	0.128
3	0.25
4	0.392

Table 4.9.2 Optimiation of glucose content by Steam distillation in STW

TIME (h)	GLUCOSE LEVEL (mg/mL)
1	0.329
2	0.584
3	0.792
4	0.893

Optimization Of Carbon Source In The Culture Medium For Bioethanol Production By Fermentation In Scw And Stw

Quantitative Analysis Of Bioethanol By Gc-Ms

Conclusion

SCW and STW are the most abundant, non- edible, agro-industrial waste of large potential feedstocks for oil extraction, bioactive compounds and bioethanol production. The interesting results of 12.5 % oil in SCW with diterpene compounds as a bioactive compound in coffee oil and 2.5 % oil in STW has its significant role in industrial applications. The anticancer activity results by cell lines for diterpene shows activity at 300µg against breast cancer has its significant role in pharmaceutical industry. Ethanol Production from the left over solid waste of (i) Coffee and (ii) Tea after oil extraction has yield factor ($Y_{P/S}$ = 0.406 g/g) and efficiency (η=78.9 %). Such results could be even improved by establishing the operational conditions that maximize the product formation. This study is a better alternate way of reusing, reducing and recycling the waste that can be implemented.

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