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Perforated Tube vs. Soxhlet Apparatus: A New Extraction Tool Confirming by Clove Sample and GC-Mass Analysis

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Abstract

In this paper, a new efficient extraction tool of bioactives from plant parts was designed, applied, and compared with Soxhlet apparatus. This tool was a Quickfit®- Pyrex® glass tube perforated easily inserted in round flask containing solvent. Clove buds in both non-grinded and grinded form were analyzed by GC-Mass. Our observations were gotten from designing and using perforated tube compared to Soxhlet apparatus for Clove buds' extraction. It is a simplest tool in designing and handling than Soxhlet apparatus that ensured more contact between Clove buds in their bag and used solvent than Soxhlet. Employing bag sewed from face mask is low cost than high quality Soxhlet - cellulose thimble. Furthermore, this sewed bag can be discharged from its content, cleaned, and reused but expensive cellulose thimble is made for one use. Perforated tube presents a good choice for efficient-short extraction time, minimum sample weight and solvent volume resulting higher quantity of Clove bioactives compared to Soxhlet. By analytical foundations, using grinded Clove gave different qualitative-quantitative analysis of the tested bioactives in the extracted solution compared to non- grinded buds. Eugenol was the major constituent in non- grinded buds while grinded powder gave 2-methoxy-3-(2-propenyl)-phenol as a maximal bioactive. This new tool may be used with less solvent quantity for better continuous extraction series however Soxhlet must be used with more than half flask volume to get fast-efficient extraction. Also, Soxhlet needs direct heating source however perforated tube may be applied in both direct heating and water bath. These notes are important in using low boiling solvent (volatile) for better extraction. Because extraction is not limited to plant parts, this new tool may be used as a general extraction tool. So to guarantee getting higher quantities of volatile constituents with easier steps, perforated tube is an excellent choice.

1. Introduction

Clove is a medicinal plant (Figure 1.) containing phenolic constituents: Eugenol and its acetyl form, and Ylangene, phytosterols: Sitosterol, Campesterol, and Stigmastanol; terpenoids such as oleanolic acid and Humilene; flavonoids: Quercetin, Rhammetin, and Kaemferol; and other constituents (Figure 2) [1]. This spice has been used as a nutritional, antioxidant, anticancer, antiviral, antifungal, and antibacterial activities besides using it in dairy products and food preservatives. It extracts by various methods such as microwave, ultrasonic, solvent, and supercritical carbon dioxide. The resulted extract is converted to capsules, complexes, and nanomaterials to treat toothache, join pain, capscaicin agonist, and antispasmodic issues [2-8].

Figure (1). Clove (Syzygium aromaticum L.).

Scientists in all over the world published huge number of research articles and reviewed many of them in extraction techniques such as Soxhlet, Clevenger or Deryng apparatus, solvent (maceration, shaking), ultrasonic – assisted, microwaves - assisted, pulse electric field, thermal deposition, percolation, pressurized liquid, steam distillation, and supercritical fluid techniques. These techniques differ in design base and output, experimental conditions such as temperature, solvent, time, etc. towards getting better extraction results in quality and quantity beside cost and eco-friendly environmental aspects [9-15].

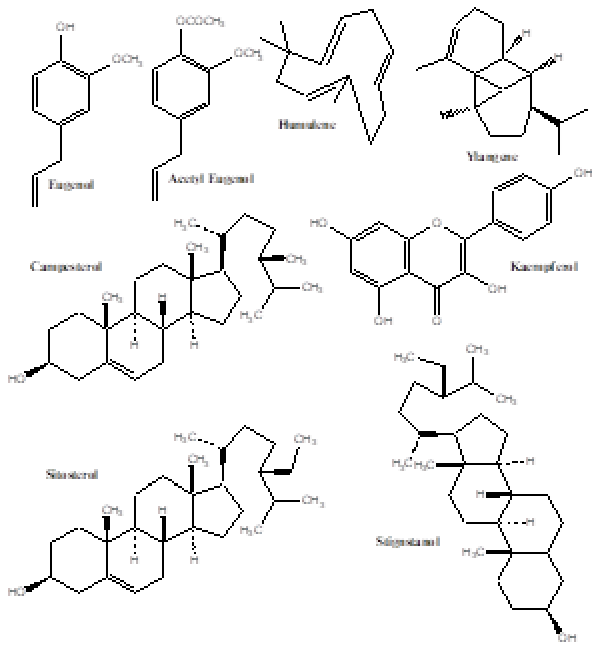

Figure (2). Main Active Constituents in Clove as in literatures [1-3].

As a general goal of any researcher in extraction subject is combining simplicity, less time–consuming, using minimal solvent volume that produce best quantity of these green materials in plant. With these important point of view, a glass tube was perforated as an easily design and inserted in round flask having solvent. The resulted solution (choosing clove buds) was analysed by gas chromatography combined with mass spectrometry then a comparison was made with Soxhlet results.

2. Experimental Procedure
2.1. Materials
Dried Clove flower buds were purchased from local Iraqi market in Baghdad city. Some of these dried Clove buds were grinded with commercial electric blender (ElAraby MX900 trade mark). Ethanol was from Hyman, England.

2.2. Instruments and Methods
Perforated extraction glass tube was designed as a new extraction tool to be used in extraction process (Figure 3.) by combing Quickfit®- Pyrex® glass tubes previously perforated to make many distributed holes was inserted in round bottom flask (Figure 4.). Other extraction technique that used in this work for comparison was Soxhlet apparatus.

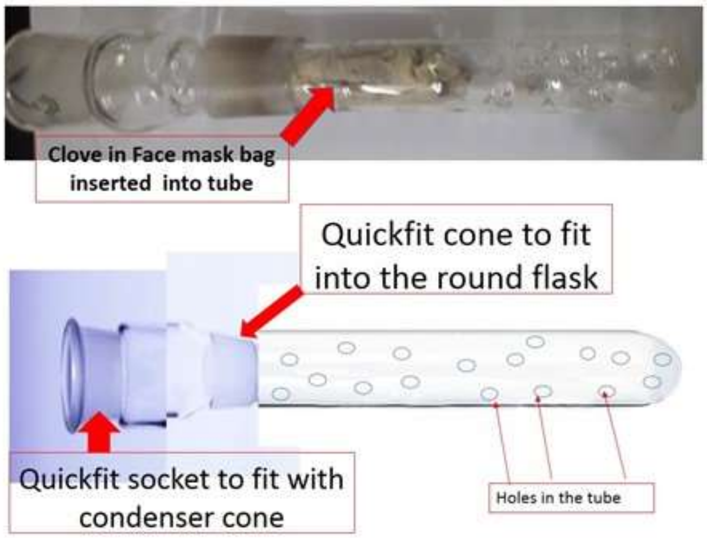

Figure (3). Perforated tube.

Gas chromatography–Mass Spectroscopy (GC-MS) in Ibn Al–Betar Centre/ Ministry of Industry and Minerals/ Iraq was chosen for identification of extracted constituents in each test. Major components of Clove extracts were analysed by using GC-Mass (Agilent 7820A, Agilent Technology, USA) equipped with HP-5ms Ultra column having (30 m length, 250 micrometres as an inner diameter, and 0.25 micrometre as a thickness).

After optimal and sensitivity conditions performing, 1 µL of Clove extract was injected where helium gas (99.99%) was the carrier gas at an initial pressure 11.933 psi. other GC conditions were type: Splitless, inlet temp. (250 °C), and injector temperature (250 °C). Results of GC-Mass analysis of all four tests (Figures (5 to 8)) were tabulated as in Tables (1 & 2.).

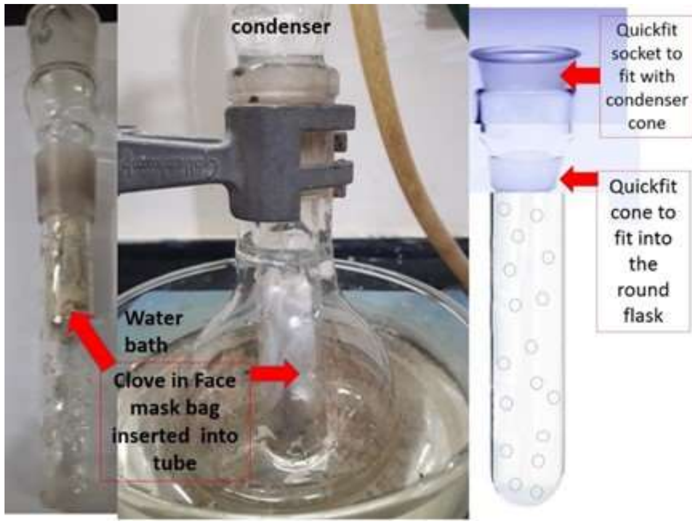

Figure (4). Perforated tube with its extraction system.

Test 1: 5gm of non-grinded Clove buds in small bag was easily inserted in the perforated tube then fixed in 250 mL flatted bottom flask. A small bag from face mask that is currently used to face Corona Virus-19 was sewed after removing the blue layer. 75 mL of absolute ethanol was used to extract bioactives, poured in round flatted flask, and heated in a water bath for 3 hours. The extracted solution was easily removed from the flask.

Test 2: Here, Test 1 conditions were repeated here with Soxhlet apparatus not new extraction tube (perforated tube) where heating was heating mantle (no water bath).

Test 3: Also, Test 1. was repeated in this test with using finely grinded Clove buds.

Test 4: Here, Test 2. was done again with grinded Clove buds.

After extraction (only Tests 1 and 2), ethanol was removed from solution by using rotary evaporator.

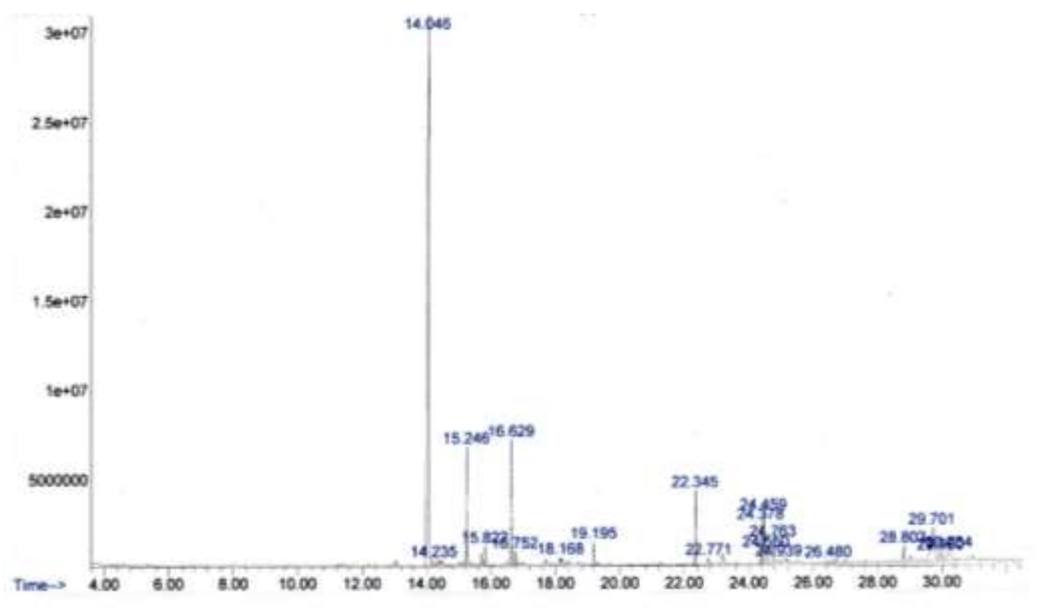

Figure (5). GC-Mass analysis of Test 1.

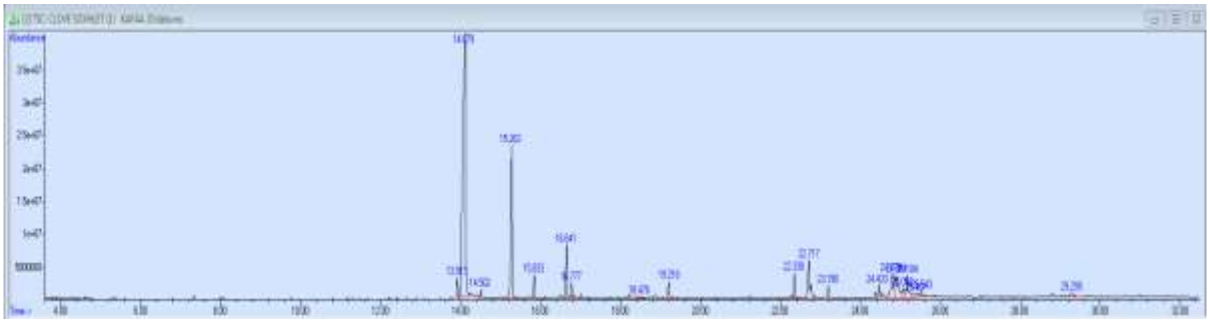

Figure (6). GC-Mass analysis of Test 2.

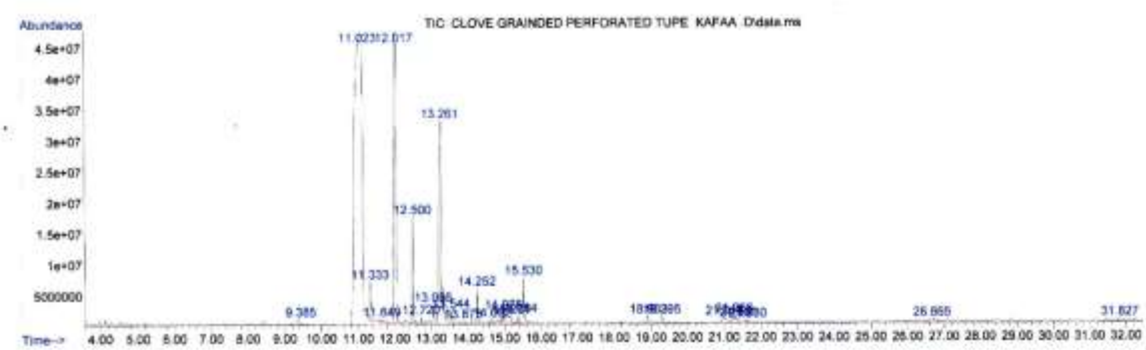

Figure (7). GC-Mass analysis of Test 3.

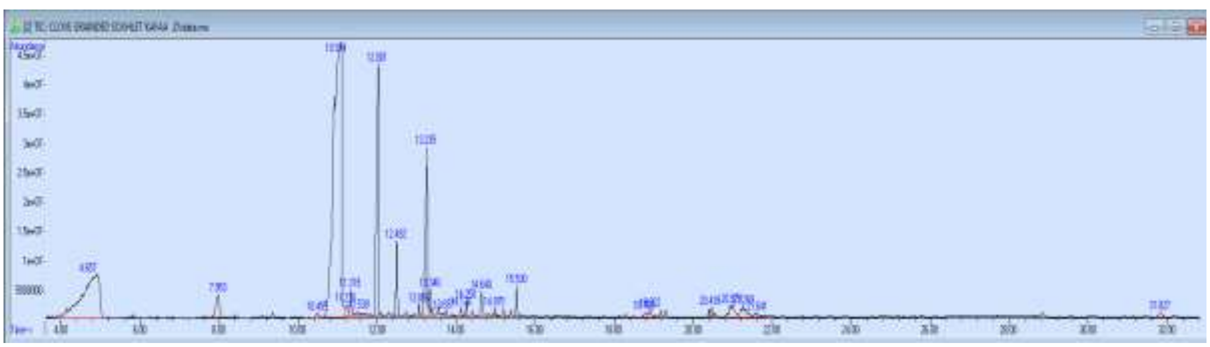

Figure (8). GC-Mass analysis of Test 4.

Table (1). GC- Mass results of major constituents in non- grinded Clove buds extraction as in Tests (1 & 2).

Soxhlet apparatus			**Perforated tube**		
RT, min	Area, %	Identity	RT, min.	Area, %	Identity
14.079	49.283	Eugenol	14.046	55.740	Eugenol
15.263	12.215	Caryophyllene	29.701	7.778	β- Sitosterol
22.717	5.913	n-Hexadecanoic acid	15.246	6.577	Caryophyllene
24.914	4.672	cis-9-Hexadecenal	16.629	6.129	Phenol, 2-methoxy-4-(2-propenyl)-, Acetate (or **Acetyl eugenol**, or eugenyl acetate)
24.778	4.432	(Z)- Heptadecanoic acid, 16-methyl-, methyl ester	22.345	3.620	Hexadecanoic acid, methyl ester
16.641	4.083	Phenol, 2-methoxy-4-(2-propenyl)-, Acetate (or **Acetyl eugenol**)	24.459	2.837	11-Octadecenoic acid, methyl ester

Soxhlet apparatus			Perforated tube		
RT, min	Area, %	Identity	RT, min.	Area, %	Identity
25.184	3.234	Octadecanoic acid	30.234	2.667	Cholest-5-en-3-ol, 24-propylidene, (3.beta.)-
22.338	2.073	Hexadecanoic acid, methyl ester	24.378	2.056	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
19.218	1.762	2',3',4' Trimethoxy acetophenone	24.763	1.408	Heptadecanoic acid, 16-methyl-, methyl ester
15.833	1.720	Humulene	19.195	1.321	2',3',4' Trimethoxyacetophenone
24.433	1.634	7-Hexadecyn-1-ol	28.803	1.295	6-Methyl-7,8-dihydro-2(1H)-pteridinone
13.913	1.458	3-Allyl-6-methoxyphenol (m-Eugenol, Chavibetol)	29.960	1.264	Stigmast-5-en-3-ol, oleate
16.777	1.245	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	15.822	1.229	Humulene
25.058	1.158	l-(+)-Ascorbic acid 2,6-dihexadecanoate	18.168	1.213	Thiophene, 2-ethyl-5-octyl-Phosphonic acid, (1-methylethyl)-,
25.407	1.056	ethylcyclopropyl)methyl] cyclopropyl]methyl]-, methyl ester	24.550	1.089	9-Octadecenoic acid, methyl ester, oleic acid methyl ester
29.299	1.032	γ.- Tocopherol	16.752	0.843	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-
23.190	0.856	Hexadecanoic acid, ethyl ester	14.235	0.813	Phenol, 2-methoxy-3-(2-propenyl)-
25.543	0.773	Octadecanoic acid, 17-methyl-, methyl ester	26.480	0.797	Benzaldehyde, 2-nitro-, diaminomethylidenhydrazone
18.476	0.752	Carbamic acid, diethyl-, methyl ester	22.771	0.676	l-(+)-Ascorbic acid 2,6-dihexadecanoate
14.502	0.646	Copaene	24.939	0.649	N-(2-Chloroethyl)benzamide

Table (2). GC- Mass results of major constituents in grinded Clove buds extraction as in Tests (3 & 4).

Soxhlet apparatus			Perforated tube		
RT, min	Area, %	Identity	RT, min	Area, %	Identity Qual.
10.970	49.40	Phenol, 2-methoxy-3-(2-propenyl)-,	11.022	58.54	Phenol, 2-methoxy-3-(2-propenyl)-,
12.00	11.28	Caryophyllene	12.017	16.90	Caryophyllene
			13.263	12.48	Phenol, 2-methoxy-4-(2-propenyl acetate (eugenyl acetate, acetyl eugenol)
13.237	7.67	Phenol, 2-methoxy-4-(2-propenyl acetate (eugenol acetate)	12.502	2.72	Humulene
12.493	2.03	Humulene	15.531	1.37	2',3',4' Trimethoxy acetophenone
20.975	1.35	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	11.333	1.03	α- Copaene
			14.250	0.92	Caryophyllene oxide
21.287	1.33	9-Octadecenoic acid, (E)-, trans-Oleic acid	21.019	0.72	cis-Vaccenic acid, ((Z)-octadec-11-enoic acid)
13.341	0.93	Naphthalene, (1S-cis)-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,	21.270	0.52	cis-13-Octadecenoic acid

Soxhlet apparatus			Perforated tube		
RT, min	Area, %	Identity	RT, min	Area, %	Identity Qual.
11.316	0.88	α- Copaene	14.977	0.50	Adamantane
14.640	0.88	2,4,6- Trimethoxy acetophenone	13.056	0.48	α- Farnesene
15.531	0.87	2',3',4' Trimethoxy acetophenone	26.662	0.48	γ- Sitosterol
			18.933	0.47	Pentadecanoic acid
			9.386	0.41	Phenol, 4-(2-propenyl)-, (Chavicol)
20.439	0.50	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	13.540	0.35	Naphthalene, (1S-cis)-1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
18.924	0.47	Octadecanoic acid			
21.642	0.45	Cyclododecanol, 1-ethenyl-			
14.250	0.40	Caryophyllene oxide	15.202	0.24	Isoaromadendrene epoxide
14.977	0.34	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.β.-ol	21.391	0.21	Oleic acid
31.829	0.32	2-methyloctacosane	21.633	0.21	3-Heptafluorobutyroxy pentadecane
			14.666	0.20	Eugenol
18.768	0.30	Pentadecanoic acid	19.296	0.19	Hexadecanoic acid, ethyl ester
13.056	0.28	α- Farnesene	31.829	0.18	2-methyloctacosane
13.696	0.28	3-Allyl-6-methoxyphenol (m-Eugenol, Chavibetol)	12.718	0.16	Muurolene
14.129	0.27	2-(3,3-Dimethyl-but-1-ynyl)-1,1,3-trimethyl-cyclopropane	13.878	0.16	trans-Isoeugenol

3. Results and Discussion

Various research articles screened extraction, analysis, and applications of Clove buds where analysis specified Eugenol was the major constituent beside caryophyllene, humulene, eugenyl acetate, cadinene, and other minor constituents. Origin of Clove plant, extraction method, time, temperature, and solvent controlled both qualitative and quantitative presence of major and minor Clove constituents [1-7, 16-20].

Recent study published by Egyptian researchers compared ethanolic extract of Clove (*Syzygium aromaticum*) buds used both maceration and sonication techniques. In this study, Gas chromatography – Triple Quad Time – Flight Mass Spectrometry specified 48 (maceration method) and 43 (Ultrasound- assisted method) bioactives. Their methodological comparison based upon time, solvent quantity, and extraction temperature. Here, sonication as a costly technique compared to maceration was more efficient in time (0.5 hr.). Also, qualitative - quantitative presence of Clove bioactives was less in maceration that is a simple method taking long time of extraction. Temperature in maceration was 27 ^{o}C not 60 ^{o}C in sonication. In conclution, sonication was more suitable in speed for Clove extraction then analysis. Eugenol derivatives were the major bioactives in both extraction methods [21].

In this study, new extraction tool was designed and used for the first time by putting in ethanolic Clove extraction as an application then GC-Mass was performed for qualitative and quantitative analysis. This new extraction tool was perforated tube (Figure 3.) in above equipped with water condenser and inserted in a round flask containing the solvent (absolute ethanol) as in Figure 4. This test was compared with known extraction technique (Soxhlet apparatus) by using the same conditions (materials and time).

Clove buds were placed in small bag then set down in the perforated tube (Figure 4.). As mentioned in experimental section, small bag was sewed from face mask that is currently used to face Corona Virus -19 after removing the blue layer to minimize any possibility of solving blue colour into extraction solution as a result of heating and ethanol presence in the flask. Both perforated tube and Soxhlet apparatus were chosen to extract non- grinded and finely grinded Clove buds.

According to many cited articles [6, 22-24], Clove contains many phenolics, tannins, flavonoids, essential oils, ... etc. including Eugenol and its derivatives, Humulene, Caryophyllene and others as main contributors in Clove traditional and biological actions. Our foundations were varied in this study from other published articles in qualitative and quantitative analysis of Clove bioactives in comparison between all four tests. Tables (1. & 2.) and Figures (5 to 8) show GC-Mass results for all four tests where several points can be summarized from these tables and experimental section as below:

- Perforated tube ensured more contact between Clove in its bag and used solvent than Soxhlet method beside it is a simplest tool in designing and handling than Soxhlet apparatus.
- Using bag sewed from face mask is low cost than high quality cellulose filter cartridge (Cellulose thimble) that used in Soxhlet extraction. Also this bag may be used in any extraction technique. Also, this bag likes cellulose thimble may be used in extraction step with no requirement for filtration step.
- Employing bag sewed from face mask is low cost than high quality cellulose filter cartridge (Cellulose thimble) that used in Soxhlet extraction. Furthermore, this sewed bag can be discharged from its contain, cleaned, and reused but expensive cellulose thimble is made for one use.
- Perforated tube was a good choice for efficient short extraction time, minimum sample weight and solvent volume.
- Perforated tube gave higher quantity of Clove bioactives compared to Soxhlet apparatus.
- Using finely grinded Clove gave different qualitative and quantitative analysis of the tested Clove bioactives in the extracted solution compared to non- grinded Clove buds.
- Eugenol was the major constituent in non- grinded Clove buds while grinded powder gave 2-methoxy-3-(2-propenyl)-phenol as a maximal bioactive.
- Perforated tube may be used with less solvent quantity for better continuous extraction series however Soxhlet must be used with more than half flask volume to get fast and efficient extraction.
- Also, Soxhlet technique needs direct heating source while perforated tube may be applied in both direct heating and water bath.
- With Soxhlet extraction, using 75 mL of absolute ethanol took more than 20 minutes for one round of extraction series.

These notes are very important in using low boiling solvent (volatile) for better extraction. So to guarantee getting higher quantities of volatile constituents with easier step, perforated tube is an excellent choice.

4. Conclusions

A new efficient extraction tool of bioactives from plant parts was designed, applied, and compared with Soxhlet apparatus. This tool was a Quickfit®- Pyrex® glass tube perforated and easily inserted in round flask containing solvent. This tube ensured the plant part was rapidly and continuously extracted by low volume of solvent. It was tested with non-grinded and grinded Clove buds giving remarkable qualitative and quantitative GC-Mass analysis results compared to Soxhlet apparatus. So, low cost, efficient perforated tube is an excellent choice to abstract bioactives in a variation presence compared to other known methods.

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