

Physicochemical assessment, characterization, antioxidant and antimicrobial potential of essential oil extracted from peel of different galgal (*Citrus pseudolimon*) cultivars

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Short Report

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Abstract

Purpose

The extraction of essential oil from citrus fruit peel is an innovative way to make use of a valuable component that is often considered waste. This research focused on extracting essential oil from three different *galgal* cultivars (Punjab *Galgal*, PBG, Haryana *Galgal*, HRG, and Himachal *Galgal*, HPG) using various methods. The extracted oils were tested for bioactive components, which were subsequently investigated for antibacterial and antioxidant properties.

Method

The peel obtained from various fruit cultivars were examined for physicochemical and phytochemical properties. The essential oils were then extracted from peel by employing different techniques and the yield was calculated to adopt best possible method. The characterization of bioactive components in essential oils was done using gas chromatography mass spectrometry method. Finally, the essential oils were studied for antioxidant and antimicrobial properties.

Results

The pH, titratable acidity, total soluble solids, moisture, color, proteins, fat, fibers, total carotenoids, chlorophyll, and ash content of fruit peel varied across different fruit varieties. Among the methods tested for essential oil extraction, the Clevenger method demonstrated superior performance compared to supercritical fluid, steam distillation, microwave-assisted, and ultrasound-assisted extraction. The essential oils extracted from the fruit peels contained varying amounts of D-limonene, α -ocimene, α -myrcene, and α -pinene, with variations observed among different cultivars. Furthermore, all essential oils exhibited good antioxidant and antibacterial properties.

Conclusion

The utilization of essential oils as antioxidant and antimicrobial agents in foods is an effective strategy for fruit industry waste valorization, and it could also best serve as a substitute for various chemical preservatives.

Statement of novelty

Galgal, an underutilized fruit belonging to the Rutaceae family, has not been thoroughly investigated for its physicochemical properties and bioactive constituents. This study aims to analyze different cultivars of *galgal* to determine their properties and explore their potential for use in various value-added products. The citrus fruit industry faces a significant challenge in managing waste generation, even in the current scenario. One of the primary waste products generated is the peel, which can be effectively utilized for waste valorization. By extracting essential oil from the peel, it can be used in food preservation, offering a sustainable solution to waste management.

Introduction

In India, citrus fruits are among the most economically significant crops. The Citrus genus, which includes approximately 140 genera and 1300 species, belongs to the Rutaceae family [1]. One of the underutilized species in the Citrus genus is *Citrus pseudolimon*, which is commonly known as *Galgal*. According to the National Horticulture Board, India 2021 report, total citrus production was 1.41 crore tones, out of which Andhra Pradesh produces 33.94 lakh tones followed by Madhya Pradesh (24.71 lakh tones), Maharashtra (18.48 lakh tones) and Punjab (12.23 lakh tones) respectively. The production of citrus products results in the creation of waste, accounting for approximately 50% of the total mass of fresh fruit. On a

global scale, the processing of citrus fruit yields approximately 10 million metric tons of waste annually. This waste comprises various components such as peels (constituting 50–55% of the overall fruit mass), seeds (comprising 20–40% of the total fruit mass), pomace, and wastewater. Citrus wastewater encompasses portions of deteriorated fruit, seeds, pulp, and peels [2]. Citrus fruits are not only commonly consumed by humans, but they also have a multitude of applications such as the extraction of valuable components or essential oils from the waste parts of the fruit (peel and pomace). This can aid in the management of waste in fruit processing. Essential oils are concentrated, aromatic liquids that are extracted from various parts of plants such as flowers, leaves, bark, roots, and fruits [3]. Essential oil is extracted by various extraction methods such as steam distillation, cold pressing, supercritical carbon dioxide extraction, and solvent extraction. These oils contain various organic compounds such as terpenes, esters, alcohols, and ketones, which give them their characteristic aroma and flavor.

Essential oils (EO) have a wide range of applications in various industries such as food, cosmetics, pharmaceuticals, and aromatherapy. They are commonly used as natural flavorings and fragrances in food products and cosmetics. Essential oils have been classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration and could serve as viable substitutes for synthetic additives [4]. They are also used in the pharmaceutical industry for their therapeutic properties and are often included in nutritional supplements. In aromatherapy, essential oils are used for their potential health benefits, which can include reducing stress and anxiety, promoting relaxation, and improving sleep quality [5].

Among the various parts of plants that are used to extract essential oils, the fruit peel is particularly popular and widely used. Essential oils extracted from citrus fruit peels, such as orange, lemon, and grapefruit, are commonly used in food flavorings, perfumes, and cleaning products due to their fresh, citrusy scent. Plants synthesize essential oils as a natural defense mechanism, and these oils are known to possess antibacterial, antifungal, and anti-parasitic properties [6, 7], which has led to a significant increase in their usage. Essential oils can be utilized as fragrances, cosmetics, and bath products, as well as for flavoring food and beverages, as incense, in household cleaning products, and for medicinal purposes.

Improper disposal of leftover citrus fruit waste after juice extraction causes environmental pollution. However, this waste can be repurposed for the extraction of essential oil, which is increasingly being used for food preservation. This study focused on the physicochemical characteristics of fruit peels from *galgal* cultivars such as Punjab *Galgal* (PBG), Haryana *Galgal* (HRG), and Himachal *Galgal* (HPG), which were used for the extraction of essential oil. Utilization of essential oil from peel is one of the best methods for waste management. The yield of essential oil was determined using different methods, and the antimicrobial and antioxidant activities of the extracted oil were also investigated. Gas chromatography mass spectrometry (GCMS) was used for the characterization of the essential oil extracted from the fruit peel.

Material and Methods

Procurement of raw material

The fruit waste (peel) of various *galgal* cultivars (Punjab, Haryana and Himachal Pradesh) were collected from Food Industry Business Incubation Centre, Punjab Agricultural University, Ludhiana, India in October to November months and stored in refrigeration condition till further use. The collected samples were then analyzed for various properties.

Physicochemical characteristics

The study involved measuring various physical characteristics of the fruit, including its peel color, thickness, and size, as well as its total soluble solids, pH, and various nutrient contents. Tools such as a digital vernier caliper (Aerospace, India), a Colour meter (Minolta CM-508d, Japan), a hand refractometer (Erma, Labline, India), and a pH meter (Mettler Toledo 5 easy plus FP20, Mumbai, India) were used for the measurements. The methods described by Ranganna [8] were used to determine the moisture, total sugars, reducing sugars, protein, fat, crude fiber, pectin, titrable acidity, ash content, and total chlorophyll and carotenoid content of the sample. The total phenol and flavonoid content were determined using the

method described by [9], while the volumetric method as described by Dinesh et al [10] was used to measure the ascorbic acid content.

Extraction and yield of essential oil

The peel was homogenized in a mixture grinder and the resulting mixture was then utilized for extracting essential oil using various methods such as Clevenger apparatus, steam distillation, microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and supercritical fluid extraction (SFE). In the MAE method, the peel was exposed to microwaves (2.45 GHz) for 10, 15, and 20 minutes, and the essential oil was extracted by Clevenger apparatus and the yield of essential oil was calculated. Similarly, the peel was treated with ultrasound for the same duration of time, using a MAC sonicator (Macro Scientific Works Pvt. Ltd, Delhi) operating at a frequency of 33 + kHz and a load of 80 watts. In case of SFE, the extraction was performed at a temperature of 45°C and a pressure of 400 bar. The flow rate of the CO₂ was maintained at 5 mL/min for 60 min, giving a total volume of 100 mL of CO₂. To prevent plugging, the restrictor temperature was maintained at 15 °C above the extraction temperature. The extracted essential oil was calculated for the yield by gravimetry method.

Characterization of essential oils by GCMS

The essential oil samples were mixed with hexane in 1:10 and 1 µL of sample was injected into GC in splitless mode. The GC-MS system used for characterizing the essential oil was equipped with a gas chromatograph (GC 1300), an autosampler (TriPlus RSH), a mass spectrometer (TSQ Duo), and a TG 5 MS column (40m, 0.15 mm, 0.25 µm) from Thermo Fisher Scientific, USA. The analysis conditions were set as follows: injector temperature at 240 °C, MS transfer temperature at 240 °C, ion source temperature at 230 °C, helium flow rate at 0.7 ml/min, initial oven temperature at 50 °C (held for 5 minutes), final temperature at 250 °C (held for 5 min), and a ramp rate of 6 °C/min. The ionization and fragmentation of the components were achieved by 70 eV electron impact. The mass filter was adjusted to a scan range of m/z 45 to 450. The results were processed using the inbuilt Xcalliber Software. The obtained peaks in chromatograms were compared and identified using NIST library.

Antioxidant activity of essential oil

Four distinct approaches were employed to measure antioxidant activity (DPPH, ABTS, FRAP and Reducing power assay). DPPH (2,2- diphenyl-1-picrylhydrazyl) test was done by the prescribed method [11]. For FRAP, ABTS and reducing power assay measurement Rumpf et al[12] method was followed.

Antimicrobial activity of essential oil

The extracted essential oil was examined for the antimicrobial activity [13] against *Staphylococcus aureus* (Gram + ve) and *Escherichia coli* (Gram -ve) by employing agar well diffusion assay. To perform the assay, Mueller Hinton Agar (MHA) plates were prepared and inoculated with 1 X 10⁸ cells/ml of each microbial strain using the spread plate technique. Wells were created in the gel using a 6mm cork corer, and a stock solution was prepared by dissolving 10mg of the extract in 10 ml of Dimethyl sulfoxide (DMSO). A volume of 0.1 ml was extracted from the stock solution and pipetted into the agar wells. The plates were then incubated at 37 °C for 24 h, and the zone inhibition diameter (mm) was measured to determine the results.

Statistical analysis

The experiments were conducted in triplicate, and the resulting data was presented as means ± standard deviation (SD). Statistical analysis was performed using SPSS software version 23. An analysis of variance (ANOVA) was conducted on all data sets, and mean values were considered significantly different at a p-value < 0.05.

Result and Discussion

Physicochemical characteristics of peel of galgal cultivars

Table 1 depicted the physicochemical properties of *galgal* cultivars (PBG, HRG, and HPG). The results showed that there were significant differences ($p < 0.05$) between the three parameters in terms of peel thickness, color (L^* , a^* , and b^*), total soluble solids (TSS), and pH. In terms of peel thickness, it was noticed highest in HPG (03.92), followed by PBG (03.57), and lowest in HRG (02.83). This indicated that HRG has a thinner peel compared to PBG and HPG. Regarding color, the L^* value was observed highest in HPG (64.83), followed by PBG (61.97), and lowest in HRG (55.52). The a^* was not significantly differ ($p < 0.05$) in case of PBG and HRG, but HPG (-13.12). The b^* value was noticed highest in HPG (61.33), followed by PBG (49.85), and lowest in HRG (49.90). The highest L^* value indicated, the colour towards lightness and $-a^*$ values indicated that the peel colour towards the green colour and b^* showed the colour towards the yellow. In terms of TSS and pH, there were no significant differences ($p < 0.05$) between *galgal* cultivars. The TSS and pH values were relatively similar, ranging from 06.52 to 06.84 and 04.01 to 04.16, respectively. Citron TSS was found to be 6.20 B and pH was noted as 2.36 and titrable acidity was calculated as 3.81 [14]. The Titrable acidity (%) values were not significantly different ($p < 0.05$) between the three samples (PBG: 7.23%, HRG: 7.42%, HPG: 7.54%) respectively.

Table 1
Physicochemical characteristics of *Galgal* cultivars (PBG, HRG and HPG)

Parameter	PBG	HRG	HPG
Peel thickness	03.57 ± 0.23 ^a	02.83 ± 0.16 ^b	03.92 ± 0.37 ^a
Colour L*	61.97 ± 0.54 ^b	55.52 ± 0.33 ^c	64.83 ± 0.55 ^a
a*	-14.51 ± 0.35 ^a	-14.49 ± 0.84 ^a	-13.12 ± 0.47 ^b
b*	49.85 ± 0.51 ^b	49.90 ± 0.61 ^b	61.33 ± 0.50 ^a
TSS	06.84 ± 0.21 ^a	06.52 ± 0.15 ^a	06.74 ± 0.11 ^a
pH	04.14 ± 0.11 ^a	04.01 ± 0.01 ^a	04.16 ± 0.02 ^a
Titrate acidity (%)	07.23 ± 0.17 ^a	07.42 ± 0.12 ^a	07.54 ± 0.11 ^a
Moisture content (%)	81.64 ± 0.12 ^c	82.44 ± 0.13 ^b	83.42 ± 0.15 ^a
Total sugars (%)	07.40 ± 0.01 ^a	06.89 ± 0.12 ^b	06.79 ± 0.11 ^b
Reducing Sugar (%)	03.82 ± 0.02 ^a	03.31 ± 0.05 ^c	03.65 ± 0.03 ^b
Crude fiber (%)	01.70 ± 0.02 ^a	01.55 ± 0.01 ^c	01.67 ± 0.01 ^b
Pectin (%)	03.52 ± 0.13 ^a	03.31 ± 0.11 ^a	03.44 ± 0.13 ^a
Protein (%)	00.13 ± 0.01 ^a	00.11 ± 0.01 ^a	00.12 ± 0.01 ^a
Crude fat (%)	04.22 ± 0.02 ^a	02.25 ± 0.04 ^c	02.87 ± 0.05 ^b
Ash (%)	02.89 ± 0.15 ^a	02.64 ± 0.12 ^{ab}	02.52 ± 0.13 ^b
Total phenols (mg GAE/g)	08.25 ± 0.17 ^a	07.26 ± 0.15 ^c	07.87 ± 0.14 ^b
Total flavonoids (mg/100g)	06.18 ± 0.11 ^c	07.23 ± 0.12 ^b	07.55 ± 0.14 ^a
Ascorbic acid (mg/100g)	39.58 ± 0.01 ^a	38.33 ± 0.13 ^c	38.65 ± 0.12 ^b
Total carotenoid (mg/g)	81.54 ± 0.10 ^a	80.24 ± 0.12 ^b	79.54 ± 0.17 ^c
Total chlorophyll	15.69 ± 0.12 ^a	15.20 ± 0.14 ^b	13.45 ± 0.12 ^c
Values are means of triplicate ± standard deviation.			
Within a row with the same lowercase letter are not significantly different at p < 0.05			

Moisture contents were significantly different ($p < 0.05$) among the samples (PBG: 81.64%, HRG: 82.44%, HPG: 83.42%) respectively. HPG was having the highest moisture content and PBG was having the lowest. Study conducted by other worker [15] showed the moisture content of lemon peel at the range of 81.23%. Total sugars content was also significantly different ($p < 0.05$) between the three cultivars (PBG: 7.40%, HRG: 6.89%, HPG: 6.79%) respectively. PBG was having the highest total sugar content and HRG and HPG was having similar lower values. Reducing sugar content of *galgal* cultivars were significantly different ($p < 0.05$). PBG (3.82%) was having the highest reducing sugar content and HRG (3.31%) was having the lowest. Crude fiber percentage was low in peel which was ~ 1% in all *galgal* cultivars. Pectin and protein of PBG, HRG and HPG were not significantly differ ($p < 0.05$). Crude fat content was found to be highest in PBG (4.22%) followed by

HPG (2.87%) and HRG (2.25%) respectively. Study conducted previously [16] revealed the fat content of lemon peel at the range of 4.2%. The ash content was highest in PBG (2.89%), followed by HRG (2.64%) and HPG (2.52%) respectively.

In terms of phytochemical content, PBG had the highest total phenols (8.25mg GAE/g), followed by HPG (7.87mg GAE/g) and HRG (7.26mg GAE/g). HRG had the highest total flavonoids (7.23mg/100g), followed by HPG (7.55mg/100g) and PBG (6.18mg/100g). PBG had the highest ascorbic acid content (39.58mg/100g), followed by HPG (38.65mg/100g) and HRG (38.33mg/100g) respectively. The total phenol content of lemon was observed by [17] in the range of 5.07 to 25.24 mg gallic acid/g. In terms of pigment content, PBG had the highest total carotenoids (81.54mg/g), followed by HRG (80.24mg/g) and HPG (79.54mg/g) respectively. PBG also had the highest total chlorophyll content (15.69mg/g), followed by HRG (15.20mg/g) and HPG (13.45mg/g) respectively. These results suggest that PBG extract has the highest content of crude fat, ash, total phenols, ascorbic acid, total carotenoids, and total chlorophyll, while HRG extract has the highest total flavonoid content. These findings could be useful for the development of plant-based food products or dietary supplements with specific phytochemical profiles.

Extraction & Yield of Essential oils

Table 2 shows the results of different techniques used for the extraction of essential oils from PBG, HRG, and HPG, respectively. The highest % yield of essential oil was obtained using supercritical CO₂ extraction with 09.40% (PBG), 07.56% (HRG), and 06.25% (HPG), respectively. This indicates that the supercritical CO₂ extraction technique was the most efficient for the extraction of essential oil, but on the other side, this technique is quite expensive because the running and maintenance costs of the equipment are high. The Clevenger extraction technique yielded 3.50% (PBG), 2.50% (HRG) and 01.92% (HPG) of essential oil, respectively, which is relatively lower than supercritical CO₂ extraction but higher than all other extraction techniques. Steam distillation, on the other hand, yielded only 00.90% (PBG), 00.81% (HRG), and 00.79% (HPG) respectively, indicating that it was not a very efficient technique for the extraction of essential oil from peel. Moreover, among the different MAE and UAE techniques used, the MAE for 20 min technique yielded the highest amount of essential oil 02.49% (PBG), 01.42% (HRG) and 01.21% (HPG) respectively. The UAE techniques yielded relatively lower amounts of essential oil compared to the MAE techniques. Steam distillation was found to be the least efficient technique for essential oil extraction. The Clevenger extraction method was found to be the most economical method of extraction because only water and heat are utilized in processing.

Table 2
Extraction of Essential Oil from peel

Sample	Technique used	Time (minutes)	% Yield of EO (PBG)	% Yield of EO (HRG)	% Yield of EO (HPG)
EO	Clevenger	60	03.50 ± 0.01 ^b	02.50 ± 0.01 ^b	01.92 ± 0.01 ^b
EO	Steam Distillation	80	00.90 ± 0.05 ^f	00.81 ± 0.02 ^h	00.79 ± 0.01 ^g
EO	MAE for 10 min	50	02.42 ± 0.04 ^d	01.31 ± 0.05 ^e	01.00 ± 0.05 ^f
EO	MAE for 15 min	50	02.45 ± 0.02 ^{cd}	01.38 ± 0.01 ^{cd}	01.10 ± 0.04 ^e
EO	MAE for 20 min	47	02.49 ± 0.05 ^c	01.42 ± 0.03 ^c	01.21 ± 0.02 ^{cd}
EO	UAE for 10 min	49	01.52 ± 0.03 ^e	01.10 ± 0.02 ^g	01.10 ± 0.01 ^e
EO	UAE for 15 min	46	01.55 ± 0.04 ^e	01.21 ± 0.01 ^f	01.18 ± 0.03 ^d
EO	UAE for 20 min	41	01.58 ± 0.06 ^e	01.35 ± 0.03 ^{de}	01.24 ± 0.02 ^c
EO	Supercritical CO ₂	70	09.40 ± 0.02 ^a	07.56 ± 0.01 ^a	06.25 ± 0.01 ^a
Values are means of triplicate ± standard deviation					
Within a coloumn with the same lowercase letter are not significantly different at p < 0.05					

Characterization of Essential oils

Table 3 illustrated the characterization of compounds present in the essential oils of three different cultivars of *galgal*, namely PBG, HRG, and HPG, using GCMS. While some compounds were common among all cultivars, others were unique to specific cultivars. For example, α -Pinene was found in all cultivars with concentrations of 0.70% (PBG), 2.65% (HRG), and 0.68% (HPG), respectively. D-Limonene was found to be the predominant compound in all samples, with concentrations of 42.94%, 56.59%, and 88.69% in PBG, HRG, and HPG, respectively. HPG had the highest content of D-Limonene, while PBG had the lowest. Linalool and Germacrene D were two other components that were common in all samples. The concentration of linalool was found to be 0.22% (PBG), 1.11% (HRG), and 2.05% (HPG), while Germacrene D was found to be in the range of 1.13%, 0.15%, and 0.09% in PBG, HRG, and HPG, respectively. Linalool was highest in HPG, while Germacrene D was highest in PBG. Additionally, some compounds were common in any two cultivars, such as α -Phellandrene, which was found in HRG (0.09%) and HPG (0.13%) only. Similarly, α -Myrcene was common in HRG and HPG, with HRG containing a good amount of α -Myrcene (27.09%) and HPG having a low amount of α -Myrcene (3.05%). α -Ocimene was found to be common in PBG (3.48%) and HPG (0.95%). ζ -Terpinene was common in PBG and HRG cultivars, with a range of 0.17% and 0.25%, respectively. Terpinen-4-ol was also found to be common in PBG (0.66%) and HRG (0.87%), respectively. In HRG, α -Terpineol was prevalent at a concentration of 0.80%, while in HPG, it was present at a concentration of 0.09%. Additionally, Geraniol and cis- α Bergamotene were detected only in HRG and HPG, with concentrations of 0.18%, 0.05% and 0.43%, 0.33%, respectively. Geranyl acetate was found in both PBG and HRG, with concentrations of 0.15% and 0.36%, respectively.

Table 3
Characterization of Essential oil from *Galgal* cultivars with GCMS

Retention time	Essential Oil					
	PBG		HRG		HPG	
	Compound Name	Area (%)	Compound Name	Area (%)	Compound Name	Area (%)
11.47			à-Phellandrene	0.09	à-Phellandrene	0.13
11.69	à-Pinene	0.70	à-Pinene	2.65	à-Pinene	0.68
12.19			Camphene	0.18		
13.08	Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene	5.24				
13.15			á-Myrcene	27.09	á-Myrcene	3.05
13.52	Trans- á-Ocimene	22.73				
14.79	D-Limonene	42.94	D-Limonene	56.59	D-Limonene	88.69
15.15	á-Ocimene	3.48			á-Ocimene	0.95
15.49	ç-Terpinene	0.17	ç-Terpinene	0.25		
16.58	Linalool	0.22	Linalool	1.11	Linalool	2.05
17.68					Limonene oxide, trans	0.15
18.00			Citronellal	0.28	Citronellal	0.04
18.29			cis-Verbenol	0.16		
18.64	Indolizine	0.20				
18.76	Terpinen-4-ol	0.66	Terpinen-4-ol	0.87		
19.07			à-Terpineol	0.80	à-Terpineol	0.09
19.76					Santalol, trans-á-	0.27
19.88	Citronellol	0.50				
19.93			Geraniol	0.18	Geraniol	0.05
20.26					Citral	0.30
20.29			Verbenol	2.98		
20.99			ç-Atlantone	4.20		
21.49					p-Mentha-1,8-dien-7-ol	0.04
22.66	Elemene isomer	0.53				
23.06	Geranyl acetate	0.15	Geranyl acetate	0.36		
23.47			2,6-Octadien-1-ol, 3,7-dimethyl-,acetate, (Z)	0.42		

Retention time	Essential Oil					
	PBG		HRG		HPG	
	Compound Name	Area (%)	Compound Name	Area (%)	Compound Name	Area (%)
23.91	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	0.59				
24.63			Caryophyllene	0.12		
24.68	N-(2,5-Dimethylphenyl)piperazine	10.42				
24.82			cis-à Bergamotene	0.43	cis-à-Bergamotene	0.33
25.12			á-Famesene	0.05		
25.37	Humulene	1.18				
25.91	Germacrene D	1.13	Germacrene D	0.15	Germacrene D	0.09
26.16					Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1S-(1à,7à,8aà)]-	1.02
26.25	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	2.28				
26.31			á-Bisabolene	0.74		
26.71					à-Maaliene	0.06
27.52	ç-Elemene	3.57			ç-Elemene	0.09
28.05	Caryophyllene oxide	0.27				
29.51	Neointermedeol	0.37			Selin-6-en-4à-ol	0.23
29.80			à-Bisabolol	0.05		
32.18	Nootkatone	0.91				

PBG contains several unique components, including Bicyclo[2.2.1]heptane, 7,7-dimethyl-2-methylene (5.4%), Trans-á-Ocimene (22.73%), Indolizine (0.20%), Citronellol (0.50%), Elemene isomer (0.53%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl) (0.59%), N-(2,5-Dimethylphenyl)piperazine (10.42%), Humulene (1.18%), (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene (2.28%), Caryophyllene oxide (0.27%), Neointermedeol (0.37%), and Nootkatone (0.91%). Overall, PBG has three dominant compounds, namely Limonene, Trans-á-Ocimene, and D,N-(2,5-Dimethylphenyl)piperazine, with concentrations above 10%. On the other hand, HRG has its own specific compounds, such as Camphene (0.18%), cis-Verbenol (0.16%), Verbenol (2.98%), ç-Atlantone (4.20%), 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z) (0.42%), Caryophyllene (0.12%), á-Famesene (0.05%), á-Bisabolene (0.74%), and à-Bisabolol (0.05%). In HRG, only two components, namely D-limonene (56.59%) and á-Myrcene (27.09%), had concentrations above 10%. In addition, HPG had only one component with a concentration above 10%, which was D-limonene. However, HPG contains some unique compounds, including Limonene oxide, trans (0.15%), Santalol, trans-á- (0.27%), Citral (0.30%), p-Mentha-1,8-dien-7-ol (0.04%), Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-,[1S-(1à,7à,8aà)]- (1.02%), à-Maaliene

(0.06%), and Selin-6-en-4-ol (0.23%). Furthermore, several studies have revealed the health benefits associated with these compounds.

D-limonene exhibits antimicrobial, antioxidant, anticancer, anti-diabetic, and antiviral effects[6, 18, 19], while α-pinene possesses antibacterial, fungicidal, and antiviral properties [20]. α-phellandrene acts as an immunity booster and has antifungal properties [21], while Myrcene has analgesic, antidiabetic, antioxidant, antifungal, and anti-inflammatory properties [22, 23]. α-elemene has anti-tumor effects, and Camphene has anti-fungal, anti-microbial, cholesterol-lowering, and antioxidant properties [24]. Caryophyllene aids in reducing pain and anxiety [25], and Humulene has anti-inflammatory, analgesic, and antibacterial properties [26]. Linalool exhibits anti-inflammatory, antioxidant, and anti-depressant effects [27]. Therefore, numerous studies suggest that essential oils have various health benefits and can be utilized in food preservation as a replacement for synthetic preservatives.

Antioxidant activity of essential oils

Table 4 provided the data on the antioxidant activity of three cultivars, PBG, HRG, and HPG, as measured by four different assays: DPPH, FRAP, ABTS, and reducing power. The results of the study showed that the PBG sample had the highest antioxidant activity as measured by the DPPH and FRAP assays, with values of 94.85% and 128.14 TE/g, respectively. In contrast, the HRG sample had the lowest antioxidant activity in these assays, with values of 88.56% and 120.64 TE/g, respectively. Interestingly, when the reducing power and ABTS assays were used, the PBG and HRG samples showed similar levels of antioxidant activity. The reducing power assay measured the ability of a compound to donate electrons and thereby reduce another substance, while the ABTS assay measured the ability of a compound to scavenge the ABTS + radical cation. Overall, the results suggested that the PBG sample may be particularly effective at scavenging free radicals, which is an important aspect of its antioxidant activity.

Table 4
Antioxidant activity of EO of different *galgal* cultivars

Sample	DPPH % DPPH Radical Scaveining Activity	FRAP TE/g	Reducing Power AAE/g	ABTS % ABTS Inhibition
PBG	94.85 ± 0.96 ^b	128.14 ± 0.98 ^a	47.73 ± 0.66 ^c	46.28 ± 0.22 ^d
HRG	88.56 ± 0.54 ^b	120.64 ± 0.64 ^a	41.35 ± 0.37 ^d	42.34 ± 0.41 ^c
HPG	86.35 ± 0.46 ^b	118.45 ± 0.35 ^a	40.56 ± 0.36 ^d	41.55 ± 0.34 ^c
Values are means of triplicate ± standard deviation				
Within a raw with the same lowercase letter are not significantly different at p < 0.05				

Antimicrobial activity of essential oils

The essential oil's antimicrobial effectiveness against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria was studied. The outcomes are illustrated in Fig. 1, indicated that there was a notably (p < 0.05) greater zone of inhibition against *S. aureus* in comparison to *E. coli*. The PBG essential oil produced a zone of inhibition ranged from 28–29 mm for *S. aureus* and 26–28 mm for *E. coli*. However, the positive control (Streptomycine) demonstrated a significantly greater zone of inhibition of 27–31 mm. The HPG essential oil exhibited a zone of inhibition ranged from 25–26 mm for *S. aureus* and 24–25 mm for *E. coli*, with the positive control showed a significantly higher zone of inhibition of 27–31 mm. On the other hand, the HRG showed a zone of inhibition against *S. aureus* and *E. coli* of 27–29 mm and 19–22 mm, respectively. The antimicrobial activity of essential oil could be due to its compounds like limonene, α-pinene, Myrcene, Camphene etc. These compounds will diffuse into the cell membrane leads to the leakage of cell wall material [13]. Our results were in accordance with the work done by [13, 28] who observed the antimicrobial properties of *Citrus nobilis* and orange peel.

Conclusion

The present study provides valuable insights into the best utilization of galgal fruit waste, highlighting its potential due to the presence of valuable constituents and their exhibited antioxidant and antimicrobial activities. The essential oil derived from galgal fruit waste showed promising properties, which can be attributed to the presence of bioactive compounds such as D-limonene, α -pinene, myrcene, and ocimene. Furthermore, the study examined various galgal cultivars and identified significant differences in their morphological and physico-chemical properties. The peel of the galgal fruit exhibited a rich phytochemical profile, including polyphenols, flavonoids, carotenoids, and chlorophyll content, thus establishing it as a valuable source of bioactive components. Considering the findings, the extraction of galgal essential oil emerges as an effective waste management approach, with potential applications in diverse food formulations. By incorporating galgal essential oil, the shelf life of food products could be extended, and it could serve as a viable alternative to synthetic preservatives. However, it is important to note that further research is necessary to evaluate the potential toxicity of the essential oil and any other harmful effects that may arise.

Declarations

Credit authorship contribution statement

Sumit Grover: Methodology, Investigation, Writing-original draft; **Poonam Aggarwal:** Resources, supervision, Writing-review & editing; **Sukhpreet Kaur:** Conceptualization, Investigation, Writing-review & editing; **Rahul Yadav:** Investigation and editing; **Anil Kumar:** Methodology, supervision and writing.

Conflict of Interest

The authors state that there is no conflict of interest

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Figures

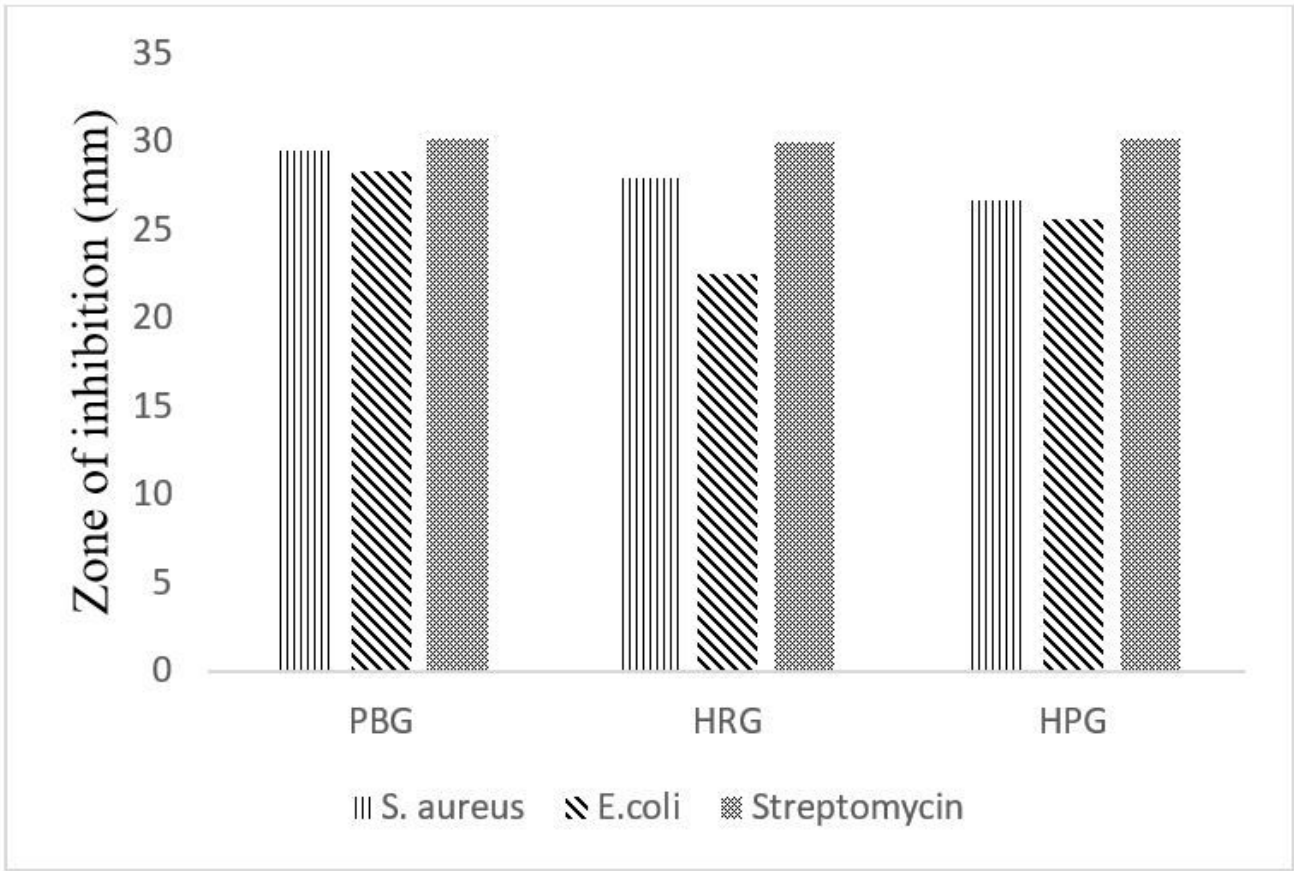


Figure 1

Antimicrobial activity of essential oils

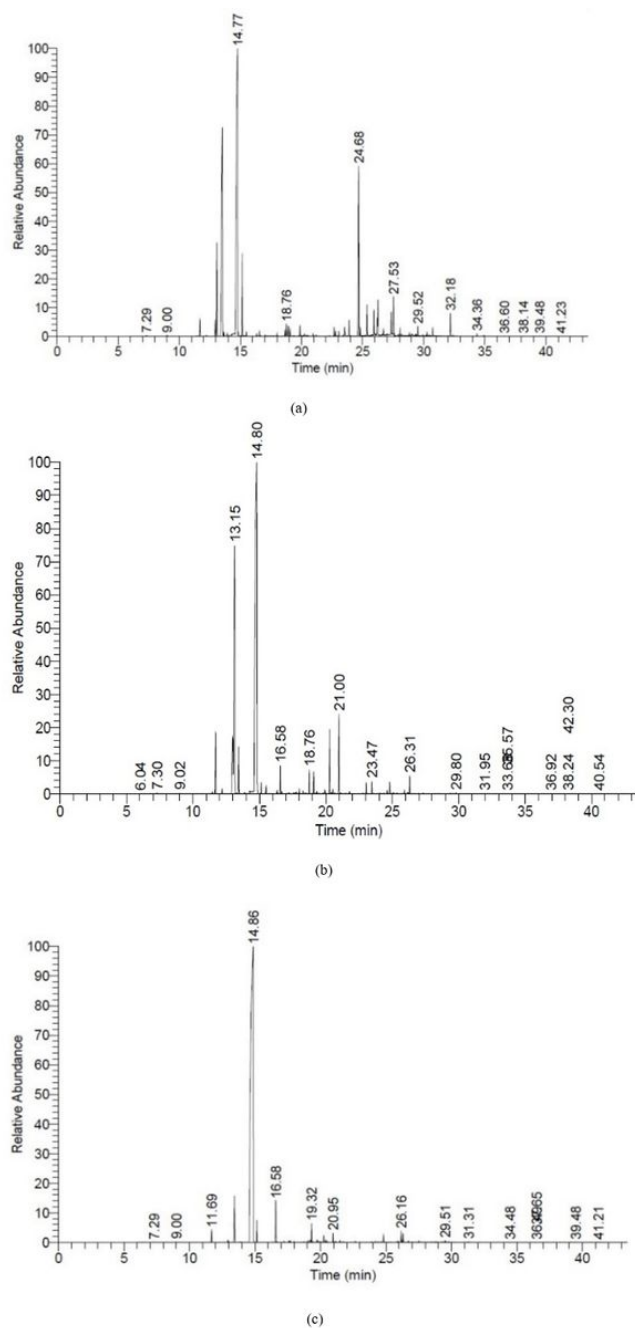


Figure 2

Chromatogram of essential oils showing bioactive constituents in a) PBG, b) HRG, c) HPG

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