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Phytochemical profiling, quantitative estimation, bioactivity studies and GC-MS analysis of fruit methanolic extract of *Kamettia caryophyllata* (Roxb.) Nicolson & Suresh

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ABSTRACT

Kamettia caryophyllata (Roxb.) Nicolson & Suresh, an endemic climber, has been traditionally used in various medicinal preparations, yet its phytochemical and pharmacological properties remain insufficiently explored. The present study aimed to investigate the phytochemical composition and biological activities of the methanolic fruit extract of *K. caryophyllata*. Preliminary phytochemical screening was carried out to identify major secondary metabolites. Quantitative estimation of phenolics and flavonoids was performed using standard spectrophotometric methods. Antioxidant activity was evaluated using DPPH radical-scavenging and phosphomolybdenum assays, and anti-inflammatory activity was assessed using nitric oxide scavenging and trypsin inhibition assays. GC–MS analysis was performed to identify specific bioactive compounds. Phytochemical screening revealed the presence of alkaloids, phenolics, flavonoids, tannins, terpenoids, and carbohydrates. Quantitative analysis showed high phenolic (169.69 mg GAE/g extract) and flavonoid (78.62 mg QE/g extract) contents. The extract showed strong antioxidant activity, with EC_{50} values of 5.20 µg/ml (DPPH) and 7.80 µg/ml (phosphomolybdenum), comparable to those of the standard ascorbic acid. Moderate anti-inflammatory activity was observed with an IC_{50} value of 170.01 µg/ml in the nitric oxide scavenging assay and 65.75% inhibition in the trypsin inhibition assay. GC–MS analysis identified several bioactive compounds, including trans phytol, methyl palmitate, methyl linoleate, oleic acid, clionasterol, lupenone, and betulol. The results suggest that *K. caryophyllata* fruits are rich in bioactive phytochemicals and possess significant antioxidant and moderate anti-inflammatory activities, indicating their potential as a natural source of therapeutic agents.

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INTRODUCTION

Medicinal plants have gained considerable importance in recent decades as valuable sources of bioactive compounds used in drug development (WHO, 2009). Since ancient times, plants have been widely used in traditional medicine systems worldwide to treat various diseases.

Medicinal and aromatic plants serve as important sources of raw materials for pharmaceutical and perfumery industries. During the course of plant evolution, numerous structurally diverse phytochemicals have developed that help plants protect themselves from insects, pests, and pathogenic microorganisms (Wink, 2008). Because of these protective properties, humans have long utilized plants as natural therapeutic agents. In fact, more than one-quarter of modern medicines contain plant-derived compounds, many of which serve as lead molecules for the development of new drugs through chemical modification and synthetic approaches (Abhishek and Avinash, 2013).

Phytochemicals are widely distributed in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, and spices, and accumulate in different parts of plants such as roots, stems, leaves, flowers, fruits, and seeds. Although these compounds are not essential nutrients required for sustaining life, they are known to possess several biological properties, including antioxidant, antimicrobial, anticancer, and immunomodulatory activities (Kavitha and Premalakshmi, 2013; Thilagavathi *et al.*, 2015). As a result, phytochemicals play an important role in preventing or managing many common diseases.

Traditional medicinal knowledge has often guided researchers in identifying plants with pharmacological potential. The classical work *Hortus Malabaricus* documents more than 2789 prescriptions used for the treatment of over 210 diseases, many of which involve plant species whose phytochemical constituents remain insufficiently explored (Mishra *et al.*, 2016). One such plant is *Kamettia caryophyllata* (Roxb.) Nicolson and Suresh, a large climber belonging to the family Apocynaceae. It is locally known as "Kametti valli" in Malayalam and is endemic to the southern Western Ghats, where it grows in evergreen and semi-evergreen forests and sacred groves (Middleton and Suddee, 2005). Traditional records in *Hortus Malabaricus* mention its use in the treatment of conditions such as spasms, epilepsy, arthritic pain, itching, scabies, cachexia, lichen, and leprosy (Manilal and Remesh, 2010).

Despite its traditional importance, the phytochemical composition and pharmacological properties of *K. caryophyllata* remain largely unexplored. Therefore, the present study was undertaken to investigate the phytochemical profile of the methanolic fruit extract of *K. caryophyllata* through qualitative and quantitative analyses and to identify bioactive compounds using GC–MS analysis. Besides that, the study aimed to evaluate the antioxidant and anti-inflammatory activities, providing scientific evidence supporting its potential therapeutic value.

MATERIALS AND METHODS

Collection and identification of plant material

Healthy fruits of *K. caryophyllata* are collected in fresh condition from the Sankulangara sacred grove located in S.N Puram, a place belonging to the Coastal Belt of Thrissur District, Kerala. The study area lies at 10.520 N, 76.210 E and has an average altitude of 2.83m (Fig. 1).

Fig. 1. A) *Kamettia caryophyllata* (Roxb.) Nicolson & Suresh Habit B) *K. caryophyllata* Fruit

Extraction of plant sample

Fresh and healthy fruits of *K. caryophyllata* is collected and washed thoroughly under running tap

water. The collected material is then air dried under shade and then powdered. Then, 100 g of the powdered plant material is placed in a Soxhlet apparatus and extracted with methanol. Subsequently, the extract is filtered, and the filtrate is then evaporated using a vacuum evaporator under reduced pressure at a temperature of <40 °C to dryness, until a constant weight is obtained. The crude dried extract obtained after evaporation is stored in desiccators for further studies.

Preliminary phytochemical analysis

Qualitative chemical tests in the methanolic fruit extracts of *K. caryophyllata* were carried out using the standard procedures described in Experimental Phyto pharmacognosy (Khadabadi *et al.*, 2013) (Table 1).

Table 1. Qualitative phytochemical screening of the methanolic fruit extract of *K. caryophyllata*

Phytochemicals	Test	Observation
Alkaloids	Dragendorff's test	Reddish-brown precipitate
	Mayers test	Cream precipitate
Tannins & Phenolics	Ferric chloride test	Blue-green to black colour
	Lead acetate test	White Preciitate
Flavonoids	Shinoda test	Orange, red or Pink colour
Glycosides	Legal's test	Pink or red colour
Terpenoids	Salkowski test	Red colour in chloroform layer with green fluorescence in acid layer
Saponins	Foam test	Stable foam for more than 15 min
Carbohydrates	Molisch's test	Red-violet ring at the interface
Proteins	Biuret test	Violet or pink colour
	Millons test	Red colour after heating

Quantitative determination of secondary metabolites

Total phenolic content

Total phenolic content of the extract was determined using the Folin-Ciocalteu method as described by Singleton *et al.* (1999). Briefly, 0.05 ml of plant extract (1 mg/ml) was mixed with 2.5 ml of 10-fold diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated at room temperature for 30 min, and the absorbance was measured at 760 nm using a spectrophotometer. Gallic acid (2–12 µg/ml) was used to prepare the calibration curve. Total phenolic content was calculated from the regression equation of the standard curve and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

Total flavonoid content

Total flavonoid content was determined using the aluminium chloride colorimetric method described by Woisky and Salatino. Briefly, 1.5 ml of extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium acetate, and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min, and absorbance was recorded at 415 nm using a spectrophotometer. Quercetin (2-12 µg/ml) was used as the standard for preparing the calibration curve. The flavonoid content was calculated using the regression equation and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

Antioxidant assay

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The antioxidant activity of methanolic fruits extracts of *K. caryophyllata* was analyzed by method described by Chang *et al.* (2001). 1ml of Plant extract of different concentrations was mixed with 3ml of 0.1mM solution of DPPH. After dark incubation for 30min in room temperature, the absorbance was measured at 517nm against methanol as blank. The reagent solution was used as control.

% of free radical scavenging activity of sample= 100×(A1-A2)/A1

A1- Absorbance of control

A2- Absorbance of sample

Phosphomolybdenum assay

The total antioxidant capacity of the methanolic fruit extract of the *K. caryophyllata* was determined by technique proposed by Prieto *et al.* (1999). 1ml of Reagent solution (28mM Sodium dihydrogen phosphate, 0.6 M Sulfuric acid, and 4 mM Ammonium molybdate) added to test tubes. Different concentration of plant extract also added to test tubes. For 90 min, the reaction mixture was kept in boiling water (95°C). Absorbance was read at 695nm against methanol as blank. 1ml of reagent solution used as control.

% antioxidant capacity= 100×(A1-A2/A1)
A1- Absorbance of control
A2- Absorbance of sample

Anti-inflammatory assay

Nitric oxide scavenging activity

The *K. caryophyllata* methanol extracts in 20µl of varying concentrations was combined with 0.5ml of 10 mM sodium nitroprusside in phosphate buffer solution (PH 7.4) and incubated for 150 minutes at room temperature (Marcocci *et al.*, 1994). Following this incubation, 0.5mL of Griess reagent (1% sulfonamide and 0.1% naphthalene ethylenediamine dihydrochloride in 2.5% orthophosphoric acid) was added to reaction mixture, and the absorbance was measured at 546nm. The inhibition of nitric oxide generation was estimated by comparing the absorbance values of the control with those of the treatments. Phosphate buffer saline was kept as a blank, and the control solution was prepared with reagent and solvent devoid of extract.

% of inhibition of nitric oxide generation= {(control OD-Treated OD)/Control OD}×100

Trypsin inhibition assay

The trypsin inhibition assay procedure of Marchetti *et al.* (1998), with slight modifications, was used here. 0.06 mg trypsin (dissolved in 1ml of 20mm Tris HCl buffer, pH 7.4) was mixed with varying concentrations of plant extract to a final volume of 2ml. after incubation for 5min at 37°C, 1ml of 0.8% Azocasein dissolved in 20mm NaHCO3, Ph 8.1 was added to the reaction mixture at 37°C, for 20 min. the reaction was stopped by adding 2ml of 10%(w/v) TCA solution. The assay mixture was centrifuged at 12000rpm for 10min. Then 2 mL of 1 M NaOH was added to the supernatant, and absorbance was measured at 440nm against the buffer as a blank. The percentage of proteinase-inhibitory activity was calculated as described.

% inhibition of denaturation= 100×(1-A2/A1)
A1- Absorbance of control
A2- Absorbance of sample

GC-MS screening for volatile bioactive compounds

GC-MS screening of methanolic fruits extracts of *K. caryophyllata* are carried out using GC Agilent Technologies (Model-5975C) system interfaced to a mass spectrometer (GC-MS) instrument (MS 7890A) employing the following conditions: column DB5-MS fused silica capillary column (30 X 0.25 mm ID X 0.25 mm film thickness, composed of 5% Phenyl, 95% Dimethyl Polysiloxane), operating in electron impact mode at 70 eV, helium (99.999%) is used as carrier gas at a constant flow of 1 mL/min, injector temperature 250°C; ion-source temperature 150°C. The oven is programmed with initial temperature 40°C for 5 min, with an increase of 5°C/min, to 280°C hold for 10 Min. Mass spectra is taken at 70 eV, a scan interval of 0.2 s and fragments are scanned from 50 to 550 Da. (Jiji and Subin, 2017). Total GC running time was 57 minutes. The constituents were identified after comparison with those available in the Computer Library (NIST ver. 2.1) attached to the GC-MS instrument and reported.

RESULTS

Preliminary phytochemical analysis

The preliminary phytochemical screening in the methanolic fruit extract of *K. caryophyllata* revealed the presence of phytochemical groups like alkaloids, terpenoids, phenolics, saponins, flavonoids, tannins, and carbohydrates, while other groups like glycosides and proteins were not detected in the methanolic extract. The details of the preliminary phytochemical screening are shown in Table 2.

Table 2. Preliminary phytochemical screening of methanolic fruit extract of *Kamettia caryophyllata*

Sl	Phytochemical group	Chemical test(s)	Results		
			R1	R2	R3
1	Alkaloids	Mayer s test	+	+	+
		Dragendroff's test	+	+	+
2	Phenolics	Lead Acetate test	+	+	+
		Ferric Chloride test	+	+	+
3	Tannins	Ferric Chloride test	+	+	+
		Lead Acetate test	+	+	+
4	Flavonoids	Alkaline reagent test	+	+	+
		Shinoda test	+	+	+
5	Terpenoids	Salkowski test	+	+	+
6	Saponins	Foam test	+	+	-
7	Glycosides	Legal's test	-	-	-
8	Proteins	Biuret test	-	-	-
		Millon' s test	-	-	-
9	Carbohydrates	Benedict's test	+	+	+
		Molisch s test	+	+	+

(+) indicate present; (-) indicate absent; R1, R2 & R3 are replicates

Table 3. Total phenolics content in methanolic fruit extract of *Kamettia caryophyllata*

Sl	Conc. of gallic acid in µg/mL	Absorbance at 760nm	Conc. of extract in µg/mL	Abs at 760nm*	Amount of total phenolic content in terms mg GAE/g (y= 0.0341x) of extract*	% Yield of phenolic extract
1	2	0.108±0.0063	50	0.29±0.013	169.69±4.98	16.97±0.49
2	4	0.162±0.041				
3	6	0.215±0.0035				
4	8	0.283±0.012				
5	10	0.310±0.003				
6	12	0.407±0.142				

*Mean of three readings ±Standard deviation

Table 4. Total flavonoids content in methanolic fruit extract of *Kamettia caryophyllata*

Sl	Conc. of quercetin in µg/mL	Absorbance at 415nm	Conc. of extract in µg/mL	Abs at 415 nm*	Amount of total flavonoid content in terms (Y= 0.017x) mg QE/g of extract*	% Yield of flavonoid content
1	2	0.029±0.0021	100	0.121±0.13	78.62±13.93	7.86±1.39
2	4	0.073±0.0516				
3	6	0.085±0.0834				
4	8	0.145±0.0226				
5	10	0.166±0.0636				
6	12	0.2075±0.0424				

*Mean of three readings ±Standard deviation

Quantitative estimation of secondary metabolites

Estimation of total phenolic content

The total phenolic content of the methanolic fruit extract of *K. caryophyllata* was determined using the Folin–Ciocalteu method, and the results are presented in Table 3. The calibration curve constructed with gallic acid showed a linear relationship between concentration and absorbance. The extract exhibited a phenolic content of 169.69 ± 4.98 mg GAE/g of extract, indicating a high concentration of phenolic compounds. The absorbance of the extract at 760 nm (0.29 ± 0.013) corresponded well with the standard curve (y= 0.0341x), confirming the reliability of the estimation. The percentage yield of phenolics was calculated to be 16.97 ± 0.49%. These results suggest that the methanolic fruit extract is a rich source of phenolic constituents, which are known for their strong antioxidant properties and potential therapeutic applications.

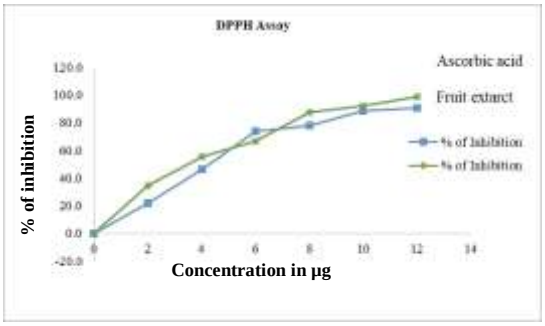
Estimation of total flavonoids content

The total flavonoid content of the methanolic fruit extract of *K. caryophyllata* was estimated using the

aluminium chloride colorimetric method, and the results are summarized in Table 4. A standard calibration curve was prepared using quercetin, which demonstrated a linear increase in absorbance with concentration. The flavonoid content of the extract was found to be 78.62 ± 13.93 mg QE/g of extract, indicating a considerable presence of flavonoid compounds. The recorded absorbance of the extract at 415 nm (0.121 ± 0.13) aligned with the regression equation (y = 0.017x). The percentage yield of flavonoids was calculated as 7.86 ± 1.39%. These findings highlight that the extract contains significant levels of flavonoids, which may contribute to its antioxidant and anti-inflammatory activities.

Anti-oxidant assay

The antioxidant activity of the methanolic fruit extract of *K. caryophyllata* was evaluated using DPPH radical scavenging and phosphomolybdenum assays. In the DPPH assay, the extract exhibited strong radical-scavenging activity with an EC_{50} of 5.20 µg/ml, comparable to the standard Ascorbic acid (EC_{50} = 5.57 µg/ml). The radical-scavenging activity increased with increasing extract concentration (Fig. 2).



Ascorbic acid, Fruit extract

Fig. 2. DPPH radical scavenging activity of ascorbic acid standard and *K. caryophyllata* methanolic fruit extract

Similarly, in the phosphomolybdenum assay, the methanolic fruit extract exhibited considerable total antioxidant capacity, with an EC_{50} of 7.80 µg/ml, while the standard Ascorbic acid showed an EC_{50} of 7.00 µg/ml. Antioxidant activity increased progressively with concentration (Fig. 3).

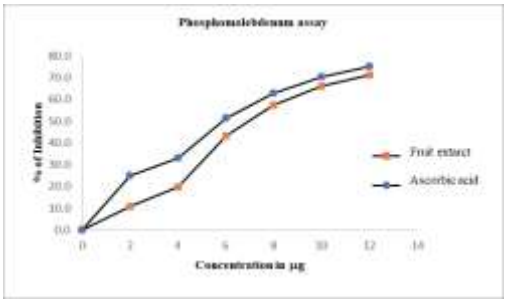


Fig. 3. Phosphomolybdenum assay of ascorbic acid standard and *K. caryophyllata* methanolic fruit extract

Anti-inflammatory assay

The anti-inflammatory activity of the extract was evaluated using nitric oxide scavenging and trypsin inhibition assays. The extract showed nitric oxide scavenging activity with an IC_{50} value of 170.01 µg/ml, indicating moderate inhibition of nitric oxide generation (Fig. 4). In the trypsin inhibition assay, the extract exhibited 65.75% inhibition, showing significant proteinase inhibitory activity (Fig. 4).

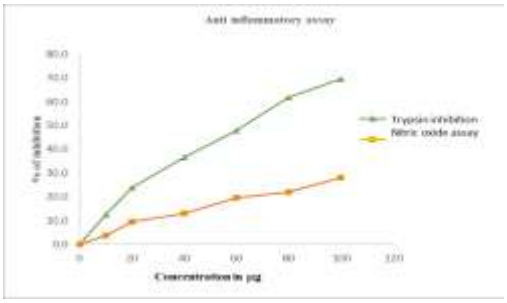


Fig. 4. Anti-inflammatory activity of the methanolic fruit extract of *K. caryophyllata*

GC-MS screening for volatile bioactive compounds

The GC-MS analysis of the methanolic fruit extract of Kamettia caryophyllata in the present investigation revealed the presence of 11 bioactive compounds (Fig. 5).

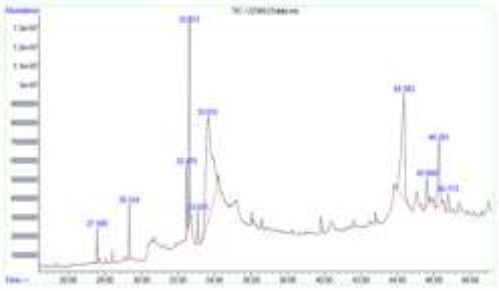


Fig. 5. GC-MS chromatogram of the methanolic fruit extract of *K. caryophyllata*

The names of bioactive compounds, peak number, retention time (RT), % of peak area, molecular formula, and molecular weight are shown in Table 5.

Oleic acid, Clionasterol, Benzyl methyl sulfoxide, and Methyl-11-octadecenoate are the major bioactive compounds identified in the GS MS studies of methanolic fruit extract based on their peak area percentage. However, most of the major and minor compounds identified in the present study are reported to exhibit attractive pharmacological activities (Table 6).

Table 5. GC-MS analysis of methanolic fruit extract of *Kamettia caryophyllata* showing the details of specific bioactive constituents

Sl	R. T	Compound name	Molecular weight	Molecular formula	Area %
1	5.304	Benzyl methyl sulfoxide	154	$C_8H_{10}OS$	13.233
2	27.568	Trans phytol	296	$C_{20}H_{40}O$	1.207
3	29.324	Methyl palmitate	270	$C_{17}H_{34}O_2$	2.314
4	32.475	Methyl linoleate	294	$C_{19}H_{34}O_2$	2.949
5	32.621	Methyl-11-octadecenoate	296	$C_{19}H_{36}O_2$	10.710
6	33.075	Methyl stearate	298	$C_{19}H_{38}O_2$	1.599
7	33.631	Oleic acid	282	$C_{18}H_{34}O_2$	35.792
8	44.363	Clionasterol	414	$C_{29}H_{50}O$	20.089
9	45.609	Lupenone	424	$C_{30}H_{48}O$	2.481
10	46.281	Methyl triacontanoate	466	$C_{31}H_{62}O_2$	7.682
11	46.773	Betulol	442	$C_{30}H_{50}O_2$	1.944

Table 6. Nature and biological activity of important compounds detected in the GC-MS analysis of methanolic fruit extract of *Kamettia caryophyllata*

Sl	Compound name	Compound nature	Bioactivity
1	Trans phytol	Terpenoid	Anxiolytic, metabolism modulating, cytotoxic, antioxidant, autophagy and apoptosis inducing, anti-inflammatory, immune modulating and antimicrobial effects (Blanco-Salas *et al.*, 2019).
2	Methyl palmitate	Fatty acid methyl ester	Anti-inflammatory, intestinal, Calcium channel (voltage-sensitive), activator Antihelmintic (Nematodes)Reductant, Antimutagenic, Antiprotozoal (Adnan *et al.*, 2019)
3	Methyl linoleate	Fatty acid methyl ester	Lipid metabolism regulator, Antisecretory, Anti-inflammatory, Reductant, Antihelmintic (Nematodes), Anti-infective (Pichersky and Gershenzon, 2002).
4	Methyl-11-octadecenoate	Fatty acid methyl ester	Antimicrobial activity (Adnan *et al.*, 2019)
5	Methyl stearate	Fatty acid methyl ester	antioxidants, cancer preventives, antidiarrheal, anti-inflammatory, pesticides, nematicides (Abdel-Hady *et al.*, 2018)
6	Oleic acid	Fatty acid	Antioxidant (Prieto *et al.*, 1999)
7	Clionasterol	phytosterol	Anticancer, anti-inflammatory, Antioxidant, antimicrobial (Dewick, 2002)
8	Lupenone	Triterpene	anti-inflammatory, anti-virus, anti-diabetes, anti-cancer (Xu *et al.*, 2018)
9	Methyl traicontanoate	Fatty acid methyl ester	Not reported
10	Betulol	Pentacyclic triterpene	Antiviral, Analgesic, Anti-inflammatory, Antineoplastic agent (Zellner *et al.*, 2009).

DISCUSSION

The present study investigated the phytochemical composition and biological activities of the methanolic fruit extract of *K. caryophyllata*. Preliminary phytochemical screening revealed the presence of several important secondary metabolites, including alkaloids, phenolics, flavonoids, tannins, terpenoids, saponins, and carbohydrates, while glycosides and proteins were not detected. The presence of these phytoconstituents indicates the pharmacological potential of the fruit extract, as many plant-derived secondary metabolites are known to contribute significantly to antioxidant, anti-inflammatory, antimicrobial, and anticancer activities.

Phytochemicals have attracted considerable attention due to their therapeutic potential and their role in the prevention and management of various human diseases. Among them, alkaloids are among the most therapeutically important plant metabolites and have wide applications in drug development. These compounds act as effective free radical scavengers and possess analgesic, antihypertensive, and bactericidal properties (Jang *et al.*, 2009; Stray, 1998). Phenolic compounds play a crucial role in plant defence mechanisms by protecting plants from microbial attack and environmental stress (Sofowora, 1993). Similarly, tannins have gained significant attention due to their antiviral, antitumour, anti-inflammatory, and anti-ulcer activities, which are largely attributed to their strong antioxidant properties (Prieto *et al.*, 1999; Shruthi, 2014). Their antimicrobial activity is attributed to their interactions with vital proteins and carbohydrates, thereby inhibiting microbial growth (Nwogu *et al.*, 2008; Bulter, 1989).

Flavonoids are another important class of plant metabolites synthesized in response to microbial infection and environmental stress. These compounds have been reported to possess strong antimicrobial activity against a wide range of pathogenic microorganisms (Dixon *et al.*, 1983). In addition, flavonoids exhibit anti-inflammatory, antiallergic, and potent anticancer activities (Ndukwe and Ikpeama, 2013). Terpenes represent another biologically significant group of phytochemicals that have demonstrated antimicrobial and anticancer potential (Piera *et al.*, 2011; Zhang *et al.*, 2005). Carbohydrates, which serve as energy reserves in plants, also play an important role in human nutrition and metabolic processes.

Plants containing carbohydrates and related compounds are often considered valuable dietary supplements as they help improve immune function and overall physiological strength.

Therefore, the presence of these phytoconstituents in *K. caryophyllata* fruits indicates their therapeutic significance.

Quantitative estimation of secondary metabolites is essential for understanding the concentration and biological relevance of phytochemicals present in plant extracts. In the present study, quantitative analysis focused on phenolic and flavonoid compounds, as these metabolites are widely recognized for their strong antioxidant potential. The methanolic fruit extract of *K. caryophyllata* showed a high content of phenolic compounds (169.69 mg GAE/g of extract) and flavonoids (78.62 mg QE/g of extract). Phenolic acids represent a diverse group of plant polyphenols produced through the shikimic acid pathway via the phenylpropanoid pathway and may also arise as by-products of the monolignol pathway or through the breakdown of lignin in cell walls (Moorman *et al.*, 1992; Croteau *et al.*, 2000; Boudet, 2007). According to Rice-Evans *et al.* (1996), phenolic compounds exhibit different antioxidant activities depending on their chemical structure and functional groups. The antioxidant potential of phenolics may also vary depending on whether they are present in free, esterified, glycosylated, or non-glycosylated forms.

Phenolic compounds are also known to influence glucose and insulin receptor functions and therefore play an important role in the management of metabolic disorders such as diabetes.

These compounds exhibit antimicrobial properties and are widely used as natural food preservatives (Naresh Kumar and Nidhi Goel, 2019). Flavonoids, another major group of polyphenolic compounds detected in the extract, are widely reported for their antioxidant, anti-inflammatory, antiallergic, and anticancer activities (Ndukwe and Ikpeama, 2013). The significant levels of phenolic and flavonoid compounds observed in the fruit extract may be contribute significantly to the biological activities reported in this study.

The antioxidant activity of the methanolic fruit extract was evaluated using DPPH radical scavenging and phosphomolybdenum assays. In the DPPH assay, the extract exhibited strong radical-scavenging

activity, with an EC_{50} of 5.20 µg/ml, comparable to that of the standard ascorbic acid (EC_{50} = 5.57 µg/ml). Similarly, the phosphomolybdenum assay demonstrated considerable total antioxidant capacity with an EC_{50} value of 7.80 µg/ml, which was close to that of the standard ascorbic acid (EC_{50} = 7.00 µg/ml). In both assays, antioxidant activity increased with extract concentration. The strong antioxidant activity observed in the extract may be because of the prescence of phenolic and flavonoid compounds, which are known for donating hydrogen atoms or electrons and neutralizing free radicals (Blois, 1958; Prieto *et al.*, 1999; Rice-Evans *et al.*, 1996).

The anti-inflammatory activity of the methanolic fruit extract was evaluated using nitric oxide scavenging and trypsin inhibition assays. Nitric oxide plays an important role as an inflammatory mediator, and excessive nitric oxide production is associated with several inflammatory disorders. The extract demonstrated nitric oxide scavenging activity with an IC_{50} value of 170.01 µg/ml, suggesting a significant inhibition of nitric oxide production. The trypsin inhibition assay showed 65.75% inhibition, indicating these extracts are having higher proteinase inhibitory activity. Proteinases are known to contribute to tissue damage and inflammatory signalling during inflammatory responses, and inhibition of these enzymes therefore represents an important mechanism in controlling inflammation (Moncada *et al.*, 1991; Aktan, 2004; Oyedepo and Femurewa, 1995).

GC–MS analysis further confirmed the presence of several biologically active compounds in the methanolic fruit extract of *K. caryophyllata*. The major compounds identified included trans phytol, methyl-11-octadecenoate, methyl palmitate, methyl linoleate, methyl stearate, oleic acid, clionasterol, lupenone, and betulol. Previous studies have reported that compounds such as trans phytol and clionasterol possess antimicrobial and antibacterial activities (Krishnamoorthy and Subramaniam, 2014; Dewick, 2002), while compounds including trans phytol, methyl stearate, oleic acid, and clionasterol exhibit antioxidant properties (Blanco-Salas *et al.*, 2019; Dewick, 2002). Furthermore, phytocompounds such as trans phytol, methyl palmitate, methyl linoleate, clionasterol, lupenone, and betulol have been reported to possess anticancer, antitumour, and anti-inflammatory activities (Abdel-Hady *et al.*, 2018; Adnan *et al.*, 2019; Xu *et al.*, 2018). The presence of these compounds in the fruit extract suggests that they may contribute to the observed biological activities.

Another observation from the GC–MS analysis was that most of the detected compounds belong to the terpenoid class, including both major and minor constituents. Terpenoids are commonly produced by plants as defence molecules against microbial pathogens and insect pests (Holopainen, 2004). Several studies have reported the therapeutic significance of terpene compounds in the treatment of human diseases such as cancer (Zhang *et al.*, 2005), microbial infections (Bakkali *et al.*, 2008), inflammatory disorders (Meratate *et al.*, 2016), and cardiovascular diseases (Wong and Menendez, 1998). Both major and minor compounds present in plant extracts may collectively contribute to biological activities through additive or synergistic effects.

Overall, the results of the present study indicate that the methanolic fruit extract of *K. caryophyllata* contains diverse phytochemicals and exhibits significant antioxidant and moderate anti-inflammatory activities. The biological activities observed may be largely attributed to the presence of phenolic and flavonoid compounds along with other bioactive constituents identified through GC–MS analysis. These findings highlight the potential of *K. caryophyllata* fruits as a valuable natural source of bioactive compounds and support their possible application in the development of therapeutic agents.

CONCLUSION

The present study revealed that the methanolic fruit extract of *K. caryophyllata* contains important phytochemicals. Quantitative analysis showed a significant presence of phenolic and flavonoid compounds. The extract exhibited significant

antioxidant and anti-inflammatory activities, likely due to the bioactive constituents present, which confer therapeutic efficacy. GC–MS analysis further confirmed the presence of several pharmacologically important compounds. These findings suggest that *K. caryophyllata* fruits may serve as a promising natural source of bioactive compounds with potential therapeutic applications.

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