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Chemical Composition and Antimicrobial Properties of Essential Oil of *Feijoa sellowiana* O. Berg. (Pineapple Guava)

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The essential oil of *Feijoa sellowiana* was extracted by hydrodistillation and tested against three strains of bacteria and fungi. It showed wide spectrum of antibacterial and antifungal activities. The more pronounced effect was noticed against the fungal strains. The essential oil showed the presence of limonene (29.29%), β-caryophyllene (27.38%), α - pinene (8.7%), β - pinene (3.11%), isocaryophyllene (1.37%), estragole (1.35%) as the major constituents.

Keywords: *Feijoa sellowiana,* essential oil, chemical composition, GC, antibacterial, antifungal activity.

Feijoa sellowiana O.Berg. Pineapple guava, (Myrtaceae) is a small tree mainly confined to Paraguay, Urugay, N. Argentina and Southern Brazil[1-3]. It is cultivated in Govt.Botanical Gardens, Ootty and Sim's Park, Conoor in South India[4]. The aerial parts were collected in November-December and authenticated at the Herbarium of Botany Department, University of Calicut where the voucher specimen (CU 88001) was deposited. This plant is cultivated for fragrant flowers and fruits which have a delicious minty pineapple flavour; herbal medicine[3,4]. Previously isolated classes of constituents were Spathulenol, β-Cadinine, β-terpineol and β- caryophyllene [5,6].

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MATERIAL AND METHODS

Fresh and mature leaves of the plant were cleaned and dried under shade. Fifty grams of the flaked powdered material was hydrodistilled in a Clevenger apparatus[7] at 100° for 4 hour. The aromatic essential oil was collected and dried over anhydrous sodium sulphate. The pure oil was transferred into small amber coloured bottles and stored at 4-6°.

Gas liquid chromatographic analysis were carried out in Perkin Elmer HS - 40 Auto system Gas chromatograph, equipped with FID and connected with a chromatograph data processor PE Nelson 1022. Neat samples of the cooled essential oil were analyzed. The GLC conditions used were as follows:

Column character: SS (Stainless Steel), SE-30 (Silicon E-30), solid phase; chemical in the

column – 100% methyl silicon gum, mesh size – 100/100, column measurements: length 6 ft, internal diameter: 2mm, carrier gas: Nitrogen, inlet pressure: 8 psi, flow rate: 30 ml/min. Temperature programmed: from 80^0 (Initial temperature) to 220° (Final temperature) at a rate of 5^0/min. Injector temperature 200° and detector temperature 300°.

The percentage composition of the oil was computed from the GLC peak areas without using correction factor. The identity of the major components was assigned by comparing their GLC retention times with those of the standards, peak enrichment by co-injection with the standards and by comparison with literature data.

Antibacterial and antifungal activity were studied by filter paper disk diffusion method[8]. The petridishes, test tubes and glasswares were washed with mild detergent and rinsed with sterile distilled water.They were autoclaved at 121° for 10 min. The media was prepared by taking 13.6g of peptone powder added to 1000 ml distilled water. Then it was poured into test tubes ie. about 5-6 ml in each test tube. These test tubes were autoclaved at 121° for 15 min and stored.

The media used for culturing bacteria was nutrient agar medium. It was prepared by adding 28g nutrient agar in 1000 ml distilled water taken in a beaker. Then it was boiled and cooled. Media was autoclaved at 121° for 15 min. Required volume of molten media was poured into sterile petridishes under aseptic condition. The bacterial strains used were *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus.* The pure samples were obtained from the Pathology department of Kerala Agricultural University, Vellayani, Thiruvananthapuram.

The pure culture obtained from the laboratory was taken and from that 1-3 loopfull of bacteria were transferred to 5 ml of peptone water in the test tube. Then it was incubated for 1 hour at 37^0 for regeneration. After one hour this was taken out and swabbed into the nutrient agar plate. Then it was kept aside. Inoculation was done under flame in highly aseptic condition, under a laminar airflow chamber.

Disc of 4 mm diameter were cut out from Whatman No. 42 filter paper. They were sterilized by autoclaving at 121° for 15 min. Then it was stored in aseptic condition in a test tube. At the time of treatment the disc was taken out from the test tube with the help of forceps. The essential oil of different concentrations were pipetted out and poured into different clean autoclaved petridish. The discs were soaked in them and covered with glass covers. It was kept for one hour so that filter paper discs will be saturated with leaf essential oil. With the help of forceps one disc from each petridish of various concentrations, which are saturated with essential oil was taken. These discs were placed on petridishes containing nutrient media possessing bacterial inoculum. The control and standard discs soaked in acetone and antibiotic Gentamycin sulphate were also placed in each petridishes containing microbial culture. Then all of these petridishes were carefully closed and kept for incubation.

The fungal medium was prepared by adding 10g agar and 65g of Sabouraud's Dextrose Agar in 1000 ml distilled water taken in a beaker. Media was boiled and cooled, then it was autoclaved at 121° for 15min. Required volume of molten media was poured into sterile petridishes under aseptic condition. For analyzing the antifungal effect of leaf essential oil of *F. sellowiana,* clinically and industrially important fungi were selected and the pure culture were obtained from the Pathology Department of Kerala Agricultural University, Vellayani, Thiruvananthapuram. They are *Aspergillus niger, Rhizopus oryzae* and *Candida albicans.* Pure cultures were used for inoculation. Different concentrations of leaf essential oil were taken and added it into petridishes which contain the nutrient agar medium. Inoculation was done under flame by platinum wire loop. Inoculated medium were labeled and kept for observation and stored. The control and standard discs soaked in acetone and antimycotic nystatin respectively were also placed in each petridishes.

RESULTS AND DISCUSSION

Essential oil with greenish yellow colour (yield 1.83% of shade dried leaves) obtained on hydrodistillation . Nineteen constituents and certain trace chemicals were identified on GLC of which the main constituents are limonene (29.29%), β-caryophyllene (27.38%), α– pinene (8.7%), β–pinene (3.11%), isocaryophyllene (1.37%) and estragole (1.35%), Table 1.

Table 1. Details of the gc analysis of leaf essential oil of *Feijoa sellowiana*

S.No.	Name of the compound	Class	Percentage yield
1.	α – pinene	Monoterpenoid	8.70
2.	β – pinene	"	3.11
3.	myrcene	"	0.16
4.	limonene	"	29.29
5.	estragole	"	1.35
6.	neryl acetate	"	0.53
7.	sabinyl acetate	"	0.60
8.	γ– terpinene	"	0.84
9.	citronellyl acetate	"	17.08
10.	α – terpineol	"	0.33
11.	methyl iso eugenol	Phenolic compound	0.79
12.	eugenyl acetate	"	0.23
13.	aromadendrene	Sesquiterpenoid	0.28
14.	germacrene	"	0.39
15.	β– caryophyllene	"	27.38
16.	β – cadinene	"	0.54
17.	iso caryophyllene	"	1.37
18.	γ – cadinene	"	0.97
19.	farnesol	"	0.31

In the present investigation high rate of sensibility to the essential oil was recorded from the measurement of zone of inhibition in bacterial and fungal growth. The soaked filter paper discs carrying different concentrations of leaf essential oil bring about the inhibition zones. The growth inhibitions were compared with the standards *viz.* gentamycin sulphate and nystatin respectively and control solvent acetone (Table 2).

Leaf essential oil would inhibit the growth of microbes reveal that the bioactive compounds are responsible for bringing about the lysis of the cellwall materials. It is also possible that the alkaloid might have brought about the inhibition of some of the enzymes involved in the metabolism of growth and development. The antigenic property of the metabolites cannot be ruled out. The bacterial cellwall possesses

Table 2. Antimicrobial activity of essential oil *Feijoa sellowiana*

Microorganisms	Zone of inhibition (in mm) * Dilution of the essential oil (*Feijoa sellowiana*) in acetone			Standards Gentamycin sulphate (40 mg/ ml)	Nystatin (50IU)
	1:0	1:1	1:2		
Bacteria					
Bacillus subtilis	24	22	18	43	
Escherichia coli	28	23	16	44	
Staphylococcus aureus	29	24	19	42	
Fungi					
Aspergillus niger	37	33	28		45
Rhizopus oryzae	41	38	33		42
Candida albicans	39	36	31		41

*Zone of inhibition including the diameter of the filter paper disk (16mm);
mean value of three independent experiments.

proteins which are the antigens. The secondary metabolites can build with these antigens and bring about immune precipitations which arrest further growth of bacteria and which may ultimately bring about a clear zone of inhibition around the disc[9 - 11].

The leaf essential oil inhibits the growth of fungi also. The components of it inhibit the growth and multiplication of fungi. The bioactive components may lead to the breaking of metabolism of fungal hyphae at molecular level. The metabolites diffused into the internal nuclei of the cell might have inhibited the protein synthetic machinery. Thus the role of secondary metabolites as a substitute for the antibiotics is established [12 - 15].

CONCLUSION

The essential oil of *Feijoa sellowiana* remarkably inhibited the growth of the tested strains of bacteria (*Bacillus subtilis, Escherichia coli* and *Staphylococcus aureus*) and fungi (*Aspergillus niger, Rhizopus oryzae* and *Candida albicans*). More pronounced inhibition was to the fungi than bacterial strains.

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