MAJOR VOLATILE CONSTITUENT OF BARK AND LEAVES OF *PSIDIUM GUAJAVA* LINN. (MYRTACEAE)

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Summary

The volatile constituents of *Psidium guajava* L. bark and leaves were identified from the essential oil obtained by steam distillation and studied by GC/MS. Nine constituents from the volatile oil of bark and leaves were identified which constituent 88.85 % and 100 % of the total amount respectively. Bark of *P. guajava* contains more monoterpenes viz. 1, 8-cineole (72.72 %) and myrcene (16.13). Leaves contain monoterpene viz. D-limonene (27.14 %) as a major constituent. It also contain six sesquiterpene as copaene (3.82 %), caryophyllene (20.75%), 4, 7, 10-cycloundecatriene (4.43 %), α-farnesene (23.25%), caryophyllene oxide (9.31%) and 1H-cycloprop (e) azule-4-ol (11.30%). Essential oil from leaves shows significant antibacterial activity against *staphylococcus aureus* and *pseudomonas aeruginosa* than essential oil from bark. Anthelmintic activity was evaluated, result reveals that essential oil from leaves shows significant activity.

Introduction

Psidium guajava is commonly known as peru or jama. This tree is cultivated nearly all over India. It is native to the Caribbean, Central America and northern South America [1]. Bark is smooth. Leaves are oblong or elliptic oblong. The flowers are white, with five petals and numerous stamens. Unripe fruits and leaves are used in treatment of dirrhoea. Stem bark and root bark are astringent, having anti-inflammatory, analgesic activity and antiseptic properties [2]. Plant shows hypoglycemic and hypotensive effects [3]. Flavonoids like quercetin and avicularin were isolated from plant. Leucocynidine, luteic and ellagic acids were isolated from stem bark. Glycoside as amritoside was isolated from plant [4]. Beta sitosterol, quercetin, gallic acid were isolated from root [5]. The aim of the present research was to study chemical composition of volatile constituent present in essential oil of bark and leaves. To evaluate antibacterial and anthelmintic activities of essential oil from bark and leaves of *P.guajava*.

Keywords: *Psidium guajava*; GC-MS; antibacterial activity, anthelmintic activity.

Material and Methods

Plant Material

Bark and leaves of *P. guajava* L. were collected from Ahmednagar district, Maharashtra, in June 2006. A voucher specimen has been deposited in the herbarium of the Botanical Survey of India, Pune under reference F. no.-Pas1.

Isolation of Volatile Oil [6]

Air-dried plant material was submitted to hydrodistillation in a Clevenger-type apparatus, affording essential oil yields of 0.2 % v/w, 0.53 % v/w, of bark and leaves respectively.

GC/MS Analysis [7, 8]

GC/MS analysis was conducted using a Shimadzu QP 5050 equipped with reference libraries using SE-52 (Mega, Legnano, Italy) cross-linked fused-silica capillary column coated with 5% phenyl-polymethylsiloxane (25 m x 0.25 mm i.d. x 0.25 μm film thickness); column temperature, 60^0C (8 min) to 180^0C at 3^0C/min, to 230^0C at 20^0C/min. Injector temperature 250^0C; Injection mode, split; split ratio 1:40; volume injected, 0.2 μL of the oil. Helium was used as a carrier, using 122.2 kPa (51.6 cm/sec); interface temperature 250^0C; acquisition mass range 40-400.

Identification and Quantification [9]

The compounds of the essential oil were identified by comparison of their Linear Retention Indices, determined in relation to a homologous series of n-alkanes, with those from pure standard of reported in literature. Comparison of fragmentation patterns in the mass spectra with those stored on databases was also preformed [10, 11]. The quantification of the components was performed on the basis of their GC peak areas.

Antibacterial Assay

The minimum inhibitory concentration (MIC) of the oil was determined using the dilution technique [12] against various microorganisms namely *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The positive antibacterial activity was established by the presence of observable turbidity after 24 hrs incubation.

Anthelmintic Activity [13]

Indian adult earthworms (*Pheretima posthuma*) were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. The earthworm *P. posthuma* were collected from moist soil and washed with normal saline to remove all the faecal matter. The anthelmintic activity was evaluated on adult Indian earthworms, *P. posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [14-15]. Four groups each containing of six earthworms of approximately equal size was released into 10 ml of desired formulation. Each group containing six earthworms of approximately equal size, released in to 10 ml of desired formulation. Each group was treated with one of the following, vehicle (5% DMF in normal saline), Albendazole (20 mg/ml), or essential oil from bark or leaves of *P. guajava (*20 mg/ml, each) in normal saline containing 5%DMF. Observations were made for the time taken to paralyze and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Results

The yield of essential oil of *P. guajava* bark and leaves obtained by stem distillation was 0.2 % v/w, 0.53 % v/w.

Mass spectra

Limonene
53 (23.54), 68 (100), 79 (26.47), 93 (26.47), 107 (17.65), 121 (17.65), 136 (14.71).
Copaene
41 (48.2), 55 (27.5), 69 (13.79), 81 (31.03), 93 (48.2), 105 (100), 119 (96.55), 133 (13.79), 147 (6.89), 161 (96.55), 189 (2.9), 204 (17.24).
Caryophyllene
41(100), 65 (34.48), 69 (58.62), 79 (51.72), 93 (65.51), 105 (37.93), 119 (24..13), 133 (55.17), 147 (20.68), 161 (24.13), 175 (6.89), 189 (13.79), 204 (3.448).

4, 7, 10-Cycloundecatriene
41 (34.48), 53 (13.79), 67 (13.79), 80 (37.93), 93 (100), 107 (13.79), 121 (24.13), 136 (3.45), 147 (17.24), 161 (3.45), 189 (3.45), 204 (6.89).
α-*Farnesene*
55 (37.83), 69 (75.86), 79 (31.03), 93 (100), 107 (24.13), 119 (27.58), 133 (10.34), 147 (3.45), 161 (13.79), 204 (3.45).
Caryophyllene oxide
55 (85.29), 69 (76.47), 79 (100), 93 (82.35), 105 (67.65), 121 (44.12), 131 (38.25), 145 (22.06), 161 (32.35), 177 (11.76), 187 (20.59), 202 (14.71).
1H-cycloprop (e) azule-4-ol
55 (70.59), 69 (70.59), 79 (72.06), 91 (100), 107 (79.41), 119 (79.41), 133 (70.59), 147 (41.18), 161 (83.82), 189 (36.76), 204 (38.24).
β-Myrcene
41 (100), 44 (65.5), 69 (48.2), 79 (37.9), 93 (48.2), 105 (31), 119 (20.68), 133 (27.5).
1, 8-Cineole
41 (100), 55 (31), 69 (51.72), 79 (24.13), 93 (48.2), 107 (20.68), 119 (20.68), 133 (6.89), 161 (6.89).

Discussion

The yield of essential oil of *P. guajava* bark and leaves obtained by stem distillation was 0.2 % v/w, 0.53 % v/w.
The results obtained from the analysis of essential oil from bark and leaves of *P.guajava* are as in table 1. Nine constituents from the volatile oil of bark and leaves were identified which constituent 88.85 %, 100 % of the total amount respectively. Oxygenated monoterpene was found to be major constituent of essential oil from bark. The essential oil from leaves was characterized by a high percentage of sesquiterpenes, amounting to 72.86 % and monoterpenes amounting to 27.14 % while essential oil from bark was characterized by a high % of oxygenated monoterpene amounting to 72.72 % and monoterpene amounting to 16.13 % (Table I).

The results of the antibacterial assay indicated that the essential oil from the leaves of *P.guajava* shows higher activity against *staphylococcus aureus* and *pseudomonas aeruginosa* than essential oil from bark (Table 2).

Anthelmintic activity was evaluated and essential oil from leaves shows significant activity than essential oil from bark (Table 3).

Table 1. Composition of volatile oil of *P. guajava* Linn. bark and leaves

Sr. no	Constituents*	Bark		Leaves	
		Retention time (min)	Amount (%)	Retention time (min)	Amount (%)
1	D-limonene	-	-	6.440	27.14
2	Copaene	-	-	10.700	3.82
3	Caryophyllene	-	-	11.258	20.75
4	4, 7, 10-cycloundecatriene	-	-	11.567	4.43
5	α- Farnesene	-	-	12.558	23.25
6	Caryophyllene oxide	-	-	12.892	9.31
7	1H-cycloprop (e) azule-4-ol	-	-	13.367	11.30
8	myrcene	13.500	16.13	-	-
9	1, 8-cineole	15.325	72.72	-	-
	Total component identified		88.85		100

Components are reported according to their elusion order.

Table-2: Antibacterial activity of essential oil from bark and leaves of *P. guajava*

Microorganisms	MIC* (mg/ml)			
	Activity	Essential oil of Psidium guajava bark	Essential oil of Psidium guajava leaves	Standard (Ampicillin)
Bacillus subtilis	-ve	Nil	NIL	0.25
Staphylococcus aureus	+ve	Nil	2.0	0.125
Escherichia coli	-ve	Nil	NIL	0.25
Pseudomonas aeruginosa	+ve	Nil	2.0	0.125

Minimum Inhibitory Concentration (MIC)

Table-3: Anthelmintic activity of essential oil from bark and leaves of *P. guajava*

Treatment	Time taken for paralysis (min)	Time taken for death (min)
EOB	6.02 ± 0.7131	13.11 ± 0.892
EOL	3.29 ± 0.018	8.31 ± 0.621
Albendazole	2.18 ± 0.536	8.54 ± 0.512
Control	-	-

EOB-essential oil from bark of *P.guajava*; EOL- essential oil from leaves of *P.guajava*

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