



***In Vitro* Antimicrobial Activity of the Extract of *Peperomia Pellucida* L. HBK (Piperaceae) Leaves Formulated as Syrup.**

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ABSTRACT

Some medicinal plants with antimicrobial activities are known to have unpleasant taste and this could affect compliance. This study is aimed at formulating the crude extract of the leaves of *Peperomiapellucida* known to possess antimicrobial activity as syrup. The antimicrobial activity of the methanol and chloroform extracts of *P. pellucida* formulated as syrup was assessed against clinical strains of bacterial and fungal organisms namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Candida albicans*. The growth of all the organisms was inhibited by the formulation obtained from methanol extract of the plant while the chloroform extract was less sensitive. The presence of sucrose in the formulation rendered the formulation pleasant to taste. The extract from the leaves of *Peperomiapellucida* L. HBK can be formulated into a pleasantly tasting oral dosage form despite its acrid taste.

Key words: *Peperomiapellucida*, antimicrobial, formulation, syrup.

INTRODUCTION

The genus *Peperomia* (Family Piperaceae) comprises more than 1000 species, including *Peperomia pellucida*, which is found in various shaded, damp habitats all over Asia and the Americas. It grows in clumps, thriving in loose, humid soils and a tropical to subtropical climate [1,2]. Common names include pepper elder (North America), little heart (Brazil) and potpopot (Oceanic) [3].

P. pellucida is an annual or short-lived perennial, erect or decumbent, freely branched, 10-50cm long, glabrous, without black glandular dots. Leaf blade palmately 5-7 veined, base truncate, rounded or cordate, apex acute to slightly acuminate, loosely flowered with matured fruiting spikes 1-2 mm in diameter. Fruits are sessile, very broadly ovoid to globose, longitudinally ribbed with ladder-like reticulations [4].

It is claimed that *P. pellucida* possesses antimicrobial activities when the crude extracts and its constituents from the plant are used [5]. Analgesic and antipyretic activities have also been established [1,6]. Flavonoids, phytosteroids, arylpropanoids, substituted styrenes and pellucidin A have been isolated from *P. pellucida* [7,8,9]. Antifungal activity has been documented for arylpropanoids such as the opiols. Other compounds, e.g. peperomins are cytotoxic or have anticancer activity *in vitro* [10]. Considering that in ethnomedicine, the extract is ingested in the treatment of gastrointestinal diseases amongst others, this study is aimed at not only establishing the antimicrobial activity of the crude extract, but also formulating the crude drug into a pleasant oral dosage form.

MATERIALS AND METHODS

Laboratory grades of petroleum ether and methanol from British Drug House (BDH) as well as granulated sugar purchased from the open market were used. The following media, Blood Agar,

Nutrient Agar and Sabouraud Agar (Oxoid) were equally provided for microbial studies,

The leaves of *Peperomia pellucida* L. HBK were collected in Ugbowo area of Benin City, Edo State, Nigeria. The plant was identified and confirmed by the plant curator of the Herbarium, Department of Pharmacognosy, University of Benin, Benin City, Nigeria, where a voucher specimen was deposited. It was dried at ambient temperatures and powdered using Moulinex mill, stored in a dry and well-stoppered bottle.

The organisms used were clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis* and *Candida albicans* from the University of Benin Teaching Hospital, Benin City.

Phytochemical studies

Chemical and Chromatographic tests were employed in the preliminary Phytochemical screening for various secondary metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex tests), alkaloids (Mayer's; Dragendorff's, Wagner's and 1% picric acid reagents), cardiac glycosides (Keller Killiani, Lieberman, Legal and Kedde tests), saponin glycosides (frothing and haemolysis tests), anthracene derivatives (Borntrager's test for combined and free anthraquinones) and cyanogenetic glycosides (sodium picrate paper test) [11, 12, 13 and 14]. Thin layer chromatography of methanol extract containing 10% H₂SO₄, shaken with chloroform and free alkaloids precipitated by the addition of excess ammonia and extracted with chloroform, on silica gel-G, activated by heating at 110 °C for 30 minutes was developed with the solvent systems Methanol : chloroform (3:7) and Acetone: water: Ammonia (90:7:3). Chromatoplates were viewed under the UV light and sprayed with Dragendorff's spray reagent and R_f values calculated.

Preparation of crude extract

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Powdered leaf material (2.0 kg) in each batch was exhaustively extracted by soxhlet extraction method using either chloroform or methanol. The extracts were concentrated *in vacuo* and stored separately in amber coloured bottles labeled A and B.

Preparation of simple and syrup solutions.

The BP method [15] was adopted to prepare simple syrup which was used as diluent to prepare concentrations of the crude extracts (A and B) varying between 25 mg/ml and 400 mg/ml. For simple solution, distilled water was used as diluent to prepare the same concentrations.

Antimicrobial screening

All the extracts were screened for antimicrobial activity by well plate method [16]. Nutrient and Sabouraud agar plates were seeded with overnight cultures of the test organism namely, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Candida albicans*. Wells, 15 mm wide, were cut in the agar plates with cork borer and 1.5 ml of the different extracts were pipetted and carefully added to the wells. The Nutrient agar plates were incubated right side up at 37 °C for 24 hr while the Sabouraud agar plate was incubated at room temperature for 48 hr. The zones of inhibition were measured and the mean of two replicates recorded.

The minimum inhibitory concentration (MIC), the lowest concentration of a compound that inhibits growth of a microorganism, was determined by the standard two-fold dilution technique using nutrient broth medium [17]. Graded concentrations of the extracts in syrup were made to achieve 25 mg/ml, 50 mg/ml, 100 mg/ml, 200 mg/ml and 400 mg/ml final concentrations in 20 ml of both Nutrient and Sabouraud agar plates. These plates were spotted with 0.1 ml overnight cultures of the test organisms.

RESULTS

Phytochemical screening of the leaves of *Peperomia pellucida* revealed the presence of alkaloids, tannins and flavonoids (Table 1). Chromatographic fingerprints were established using Thin-layer techniques (Table 2), detecting with Dragendorff for the presence of alkaloidal compounds.

Methanol extract of *P. pellucida* and Gentamycin, the reference standard, showed antimicrobial activity against the tested organisms. The chloroform extract was not as effective as the methanol extract (Table 3). Table 4 shows the sensitivity of the syrup samples prepared using different solvent systems. It therefore indicates that solvent system used in the extraction of the secondary plant metabolites is a factor in the formulation of medicinal plants into syrup. The MIC of the methanol extract formulated as syrup was also determined (Table 5).

Table. 1: Preliminary phytochemical screening of *Peperomia pellucida* leaves.

Classes of secondary metabolites	Inference
Alkaloids	
Dragendorff's reagent	+
Wagner's reagent	+
Mayer's reagent	+
1 % picric acid reagent	+
Anthracene derivatives	
Combined anthraquinones	-
Free anthraquinones	-

Solvent Systems	No. of spots	Colour in daylight	Colour in UV	Colour after spraying with Dragendorff	R _f value
Methanol: chloroform (3 : 7)	1	Colourless	Light green fluorescent	Reddish brown	0.30
Acetone: water: ammonia (90: 7: 3)	1	Colourless	Light green fluorescent	Reddish brown	0.65

Tannins

Phenazone test	+
Iron complex test	+
Formaldehyde test	+
Modified iron complex test	+

Flavonoids +

Saponin glycosides

Frothing test	-
Haemolysis test	-

Cardiac glycosides

Keller killiani test	-
Lieberman's test	-
Legal test	-
Kedde test	-

Test organism	Chloroform extract of <i>P. pellucida</i> (mm)	Methanol extract of <i>P. pellucida</i> (mm)	Gentamycin (10 µg/ml) (mm)	Solvent Aq. Methanol (mm)
<i>Staphylococcus aureus</i>	0	9	15	0
<i>Escherichia coli</i>	0	4	11	0
<i>Pseudomonas aeruginosa</i>	2	5	13	0
<i>Klebsiella pneumonia</i>	1	5	12	0
<i>Bacillus subtilis</i>	3	6	13	0
<i>Candida albicans</i>	0	8	3	0

Cyanogenetic glycosides

Sodium picrate test paper	-
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Key: - = absent; + = present

Table 2: Chromatographic results

Thin layer chromatography

Table 3: Antimicrobial screening of *Peperomia pellucida* leaves at 100 mg/mL.

The diameter recorded is the diameter of the zone of inhibition less the diameter of the cup (15 mm).

Table 4: Sensitivity of syrup samples of *Peperomia pellucida* leaves on Test Organisms at 100 mg/mL.

Test organism	Chloroform extract of <i>P. pellucida</i> in syrup 80 %w/v (mm)	Methanol extract of <i>P. pellucida</i> in syrup 80 %w/v (mm)	Syrup (mm)
<i>Staphylococcus aureus</i>	0	7	0
<i>Escherichia coli</i>	0	4	0
<i>Pseudomonas aeruginosa</i>	2	3	0
<i>Klebsiella pneumonia</i>	0	5	0
<i>Bacillus subtilis</i>	1	7	0
<i>Candida albicans</i>	0	0	0

The diameter recorded is the diameter of the zone of inhibition less the diameter of the cup (15 mm).

Table 5: Minimum inhibitory concentration MIC ($\mu\text{g/mL}$) of the methanol extract of *Peperomia pellucida* leaves formulated in syrup.

Test organism	MIC ($\mu\text{g/mL}$)
<i>Staphylococcus aureus</i>	50

<i>Escherichia coli</i>	50
<i>Pseudomonas aeruginosa</i>	25
<i>Klebsiella pneumonia</i>	50
<i>Bacillus subtilis</i>	50
<i>Candida albicans</i>	25

Values are mean (n = 5)

DISCUSSION AND CONCLUSION

The antimicrobial activity of the leaves of *Peperomia pellucida* had previously been reported [5 and 18]. This study has equally confirmed such findings. The inhibition of growth of the organisms by the extracts can be attributed to the presence of biologically active, complex organic chemicals (secondary plant metabolites) in their tissues [19]. Plants containing alkaloids and/or phenolic compounds such as tannins and flavonoids have been known to possess antimicrobial activities [20,21]. Alkaloids, tannins and flavonoids were present in *Peperomia pellucida*.

The antibacterial activities of the methanol extract and methanol extract formulated as syrup against the gram negative organisms was very significant, in the sense that gram negative organisms are usually known to be less sensitive to the standard antibacterial agents because they possess a more sophisticated cell envelop [22].

Sucrose, a sweetening agent and main content of syrup, has been used to flavour the extract, and so a pleasantly tasting oral dosage form of the extract, which can easily be acceptable to children, was formulated. Formulations made from medicinal plants can gain the confidence of orthodox health practitioners when there are scientifically

established proofs of their claimed efficacies. On the basis of the overall results from our investigations, the use of the crude extracts of *P. pellucida* in the treatment of infections by traditional practitioners is justified. Extracts of *P. pellucida* L (HBK) formulated as simple syrup have been found to possess similar antimicrobial activity as the crude product.

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