UNED Research Journal
Cuadernos de Investigación UNED

Chemical screening and biological activity of extracts from *Ficus pumila* (Moraceae) and *Phthirusa stelis* (Loranthaceae)

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Received 29-IX-2023 ● Corrected 16-II-2024 ● Accepted 25-II-2024
https://doi.org/10.22458/urj.v16i1.5008

ABSTRACT. Introduction: The plants *Ficus pumila* and *Phthirusa stelis* are emerging as natural product sources. **Objectives**: To identify chemical compounds, antibacterial and antifungal activity, and toxicity against *Artemia* sp. and *Aedes aegypti* larvae. **Methods**: We tested methanol and propanol extracts from fruits, leaves, and stems. **Results**: *F. pumila* had flavonoids, tannins, and polyphenols; *P. stelis* had flavonoids, tannins, anthraquinones, triterpenes, and polyphenols. The isopropanol stem and ethanol leaf extracts of *F. pumila* showed significant antibacterial activity, the former a 10mm zone of inhibition against *B. subtilis*; the latter, 11 mm against *S. aureus*. The ethanol leaf and stem extracts of *P. stelis* had inhibition against *S. aureus* and *B. subtilis:* 10mm each the leaf; 11 and 10mm, respectively, the stem. The isopropanol leaf extract of *P. stelis* was also highly effective against *E. coli* (15mm). Most extracts were cytotoxic against *Artemia* sp.; ethanol and propanol leaf extracts of *P. stelis* had positive LC_{50} values, particularly the propanol leaf and stem extracts of *P. stelis* (0,01 and 0,02µL/mL); the ethanol leaf extract of *P. stelis* (0,01µL/mL) and the propanol fruit extract of *F. pumila*. None of the extracts inhibited fungal growth or affected *A. aegypti* larvae. **Conclusion:** *F. pumila* and *P. stelis* have potential for therapeutic use.

Keywords: Probit, Logit, pharmacologicals, natural products, bioactive compounds.

RESUMEN. "Tamizaje químico y actividad biológica de extractos de *Ficus pumila* (Moraceae) y *Phthirusa stelis* (Loranthaceae)". **Introducción:** Las plantas *Ficus pumila* y *Phthirusa stelis* están emergiendo como fuentes de productos naturales. **Objetivos:** Identificar compuestos químicos, actividad antibacteriana y antifúngica, y toxicidad contra *Artemia* sp. y larvas de *Aedes aegypti*. **Métodos:** Probamos extractos de metanol y propanol de frutas, hojas y tallos. **Resultados:** *F. pumila* tenía flavonoides, taninos y polifenoles; *P. stelis* tenía flavonoides, taninos, antraquinonas, triterpenos y polifenoles. Los extractos de tallo de isopropanol y hoja de etanol de *F. pumila* mostraron actividad antibacteriana significativa, el primero una zona de inhibición de 10mm contra *B. subtilis*; el segundo, 11mm contra *S. aureus*. Los extractos de hoja y tallo de etanol de *P. stelis* mostraron inhibición contra *S. aureus* y *B. subtilis*: 10 mm cada uno en la hoja; 11 y 10mm, respectivamente, en el tallo. El extracto de hoja de isopropanol de *P. stelis* también fue altamente efectivo contra *E. coli* (15mm). La mayoría de los extractos fueron citotóxicos contra *Artemia* sp.; los extractos de hoja de etanol y propanol de *P. stelis* tuvieron valores CL_{50} positivos, particularmente los extractos de hoja y tallo de propanol de *P. stelis* (0,01 y 0,02µL/mL); el extracto de hoja de etanol de *P. stelis* (0,01µL/mL) y el extracto de fruta de propanol de *F. pumila*. Ninguno de los extractos inhibió el crecimiento fúngico ni afectó las larvas de *A. aegypti*. **Conclusión:** *F. pumila* y *P. stelis* tienen potencial para uso terapéutico.

Palabras clave: Probit, Logit, productos farmacológicos, productos naturales, compuestos bioactivos

Since ancient times, plants have been the subject of inquiry aimed at discovering chemical compounds that can be used to create new drugs and other substances. This has led to the development of an entire industry and has presented pharmacologists with a significant challenge. Indeed, about 52,0% of drugs derived from natural products mimic those natural products or are synthesized using a pharmacophore derived from them (Rincón, 2014). By combining information obtained from medicinal flora users, such as traditional experts and communities with chemical and pharmacological studies, there is no doubt that natural products have multiple benefits in treating various diseases (Ferreira et al., 2019).

Certain organisms create natural products in response to external conditions like water stress, heat, superpopulation, and radiation. These natural products act as chemical signals that protect them from herbivores, pests, pathogens, and symbiosis (Malec & Pomilio, 2003).

This paper focuses on the study of two plant species: *Ficus pumila* L. (Moraceae) and *Phthirusa stelis* (L.) Kuijt (Loranthaceae). Both have bioactive components that can be used to prevent and treat diseases.

Ficus is a large genus that comprises approximately 800 species, most of which have a high ornamental and ecological value (Huang et al., 2022). *F. pumila* has long been used as a functional plant in East Asia. Its fruit is a dietary ingredient in Japan and some regions of China. It contains biologically active compounds such as phenolic acids, flavonoids, terpenes, alcohols, and steroids (Qi et al., 2021) that can be extracted from its stems, leaves, flowers, and fruits. The plant is non-toxic and has a wide range of therapeutic properties such as hepatoprotective, dermo protective, and nephroprotective benefits (Shim et al.,2022) as well as antioxidant, anti-inflammatory, antibacterial, antitumor, hypoglycemic, and cardiovascular protective effects.

P. stelis is commonly used in Venezuelan traditional medicine for a variety of purposes. It is effective in treating impetigo, a bacterial skin infection (Bello, 2017), urinary retention (Clement et al., 2015), uterine cysts, gastrointestinal and bronchial ulcers, hemorrhage, pneumonia, and general fatigue. It is also used as an abortifacient (Instituto de Investigaciones de la Amazonía Peruana [IIAP], 2010), a hypotensive agent (López et al., 2016), and as a medication for cancer, pain relief, and inflammation. It is used as an ingredient in Sitz baths (Van-Andel & Van't-Klooster, 2007) and as an agent to treat cuts, wounds, and burns (Espitia & Sarmiento, 2016).

Unfortunately, most of these compounds often exhibit systemic toxicity and side effects. Considering this, it was necessary to test their potential to kill microcrustaceans in the larval stage (nauplii) as compared to other tests (*in vitro* cells of yeast strains, zebra fish, rodents, among others). This method provides a convenient starting point not only to conduct cytotoxicity studies but also to detect the general toxicity of synthetic, semi-synthetic, and natural products. It is simple, rapid, inexpensive, and easy to perform (Ntungwe et al., 2020; Santos et al., 2022).

In recent times, there has been a significant increase in the number of diseases transmitted by microorganisms (bacteria and fungi) in Venezuela. Dengue, yellow fever, Zika, and other diseases by vectors like *A. egypti* are also on the rise. Evaluating the bioactive potential of plants such as *F. pumila* and *P. stelis* would ensure the benefit of all. Their toxic effects against nauplii or *Artemia* sp. may also be gauged.

MATERIALS AND METHODS

Specimens of *F. pumila* and *P. stelis* were collected in Guaracayal, Bolívar Municipality and Muelle de Cariaco, Ribero Municipality, respectively, in Sucre State, Venezuela. The identification process was carried out under the guidance of botanical specialist Jesús Bello, M.Sc., at the Isidro Ramón Bermúdez Romero Herbarium (IRBR), Biology Department of the Universidad de Oriente, Sucre. Species identification followed the taxonomic criteria outlined in Flora de Venezuela,

employing dichotomous keys and morphological descriptions to accurately determine their scientific names.

Sample Treatment: Fruits, leaves, and stems of *F. pumila* and *P. stelis* were dried in the shade at room temperature. They were then pulverized, crushed, and mixed with isopropanol and ethanol; the mixture was shaken twice a day for 10 days at room temperature following the recommendation of Carrión and García (2010). The extracts were then concentrated under reduced pressure, using a Buchi 461 rotary evaporator to eliminate the solvent and obtain the crude extracts.

Biological Tests and Phytochemical Analyses: The bioactivity of ethanol and isopropanol extracts obtained from the fruits, leaves, and stems of both species was evaluated for antibacterial activity (Bauer et al., 1966 modified by Estaba, 1986). Paper disks measuring 5mm in diameter were impregnated with 10μL of the isopropanol and ethanol extracts with a concentration of 40mg/mL of the samples to be tested, equivalent to 0,4mg/disk. Counterpart disks impregnated with 10μL of solvent were used as negative control and ampicillin/sulbactam antimicrobial susceptibility disks (25μL) as positive control. Gram-negative *E. coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 51470), as well as Gram-positive *Staphylococcus aureus* (IBE-Doc-19) and *Enterococcus faecalis* (WHO 14) were tested.

Antifungal activity was assessed using the disk diffusion method (Madubunyi, 1995). Twenty-five microliters of the sample solution, containing a concentration of 40mg/mL were carefully dispensed onto sterile disks measuring 5mm in diameter. Subsequently, each disk was impregnated with 10μL of the extract solution undergoing testing.

Fluconazole antimicrobial susceptibility disks (25μg) were used as a positive control, and disks impregnated with 10μL of solvent were used as a negative control. The following fungal strains were tested: *Aspergillus niger*, *Candida albicans*, *Penicillium hirsutum*, *Rhizopus oryzae*, and *Fusarium moliniforme*.

To evaluate the toxic activity of the samples against *Artemia* sp. (Meyer et al., 1982), 50mg of each sample was dissolved in 0,5mL of solvent and 4,5mL of filtered seawater, resulting in a standard solution with a concentration of 10,000μg/mL. Successive dilutions of the standard solution were prepared (1000, 100, 10, 1, 0,1, and 0,01μg/mL) for testing.

The larvae of *A. aegypti* were collected in Cumanacoa, Montes municipality of Sucre State, following the methodology recommended by Bazán et al., 2011. Larvicidal activity against *A. egypti* larvae was evaluated by using bioassays to test the effectiveness of different concentrations. Standard bioassays were undertaken dissolving 50mg of each extract in 5mL of the tested solvent, resulting in a solution with a concentration of 10,000mg/mL. Ten fourth-instar *A. aegypti* larvae (Nelson, 1986) were placed in 5mL of each solution obtained from the crude extracts, and four replicates were carried out for each treatment using a total of 200 larvae per extract. Ten additional larvae in quadruplicate were used in each control for each concentration, and mortality readings were taken at 24 hours. Successive dilutions were prepared from the standard solution (1000, 100, 10, 1, 0,1, and 0,01mg/mL).

Four methods were used to analyze the data: binomial, moving average, probit, and logit with reliability limits of 95%, as described in standard bioassays toxicity protocols for aquatic species (Rodríguez & Esclapés, 1995).

A phytochemical study of alkaloids, flavonoids, tannins, cyanogenic glycosides, anthraquinones, saponins, coumarins, polyphenols, sterols, and triterpenes was conducted following the methodology recommended by Marcano and Hasegawa (2018).

RESULTS

Flavonoids, tannins, and polyphenols were identified in the isopropanol and ethanol leaf, stem, and fruit extracts of *F. pumila*. Sterols and triterpenes were also detected in the *F. pumila* isopropanol extracts of leaves and stems, respectively. In 2021, Qi et al. observed the presence of flavonoid-derived compounds in methanol and ethanol extracts from the leaves and stems but not from fruit. Similarly, Soto and Rosales (2016) also effectively extracted tannins, phenols, and terpenoids using water and ethanol as solvents.

Despite the limited availability of data regarding the chemical composition of *P. stelis*, we found that both isopropanol and ethanol extracts derived from the stems and leaves of this species were positive for flavonoids, tannins, anthraquinones, triterpenes, and polyphenols. We compared these findings with those reported for species of the same family.

For comparison, chemical compounds have been identified in several mistletoe species, including *Phragmanthera capitata* a polysaccharide was structurally identified in the cell wall of the leaves, along with pectin and hemi cellulosic polysaccharides (Aboughe et al., 2009); *Loranthus tanakae* Four antitumor flavonoid rhamnopyranosides were identified: rhamnetin 3-O-α-L-rhamnoside, quercetin 3-O-α-L-rhamnoside, rhamnocitrin 3-O-α-L-rhamnoside, and kaempferon 3-O-α-L-rhamnoside (Kim et al., 2004); *Taxillus yadoriki* fatty acids, phytosterol, phytosterol glucoside, quercetin, avicularin, and taxillusin were found (Fukunaga et al., 1989); *Dendrophthoe falcata* three new triterpenes were isolated, to wit: 3β-acetoy-1β-hydrozy-11α-methoxy-olean-12ene. Five known triterpenes, two flavonoids, and gallic acid were also reported (Mallavadhani et al., 2006).

Antibacterial activity: Considering the scale of Monks et al. (2002), the extracts showed weak antibacterial activity. The ethanol extract of *F. pumila* exhibited zones of inhibition on *B. subtilis* (10mm) and *E. coli* (7mm), and the ethanol extract of *F. pumila* leaves showed effectiveness against *S. aureus* (11mm).

The ethanol extract of *P. stelis* leaves exhibited activity against *S. aureus* (10mm), *B. subtilis* (10mm), and the stem extract from the same plant showed similar results in both bacteria, with zones of inhibition of 11 and 10mm, respectively. The propanol leaf extract of *P. stelis* also showed activity against *E. coli* (10mm).

Antifungal activity: In this study, it was found that none of the extracts from *F. pumila* and *P. stelis* showed any growth inhibitory effects against the fungi tested, and therefore, the extracts are deemed to be harmless against these fungal species. Despite our negative results, Noronha et al. (2014) did report antifungal activity of *F. pumila* extracts against *C. albicans* and *Saccharomyces cerevisiae* yeasts.

Toxic activity against *Artemia* sp.: Most of the *P. stelis* extracts demonstrated notable toxicity against *Artemia* sp. The ethanol leaf extract exhibited positive LC_{50} results at 0,01μg/mL, alongside the Isopropanol leaf extract at the same concentration (0,01μg/mL., Probit and Logit), and the propanol stem extract at 0,02μg/mL (Probit). Additionally, the Isopropanol fruit extract of *F. pumila* showed significant toxicity, with LC_{50} values of 0,44 and 0,62μg/mL (Moving Average and Probit). These findings were derived using the Moving Average method, as described by Stephan (1977), which provided a precise probability range. Tables 1 and 2 show the results in LC_{50} for isopropanol and ethanol extracts of *F. pumila* and *P. stelis*.

Table 1. Mean lethal concentration of ethanol and isopropanol extracts of *P. stelis* on *Artemia* sp. nauplii.

Extracts	Method	LC_{50} (µg/mL)	Confidence limit (95%) Lower	Upper
EITPS	Binomial	*****	*****	*****
	Moving Average	*****	*****	*****
	Probit	0,02	0,00	0,18
	Logit	0,01	0,00	0,26
EIHPS	Binomial	*****	*****	*****
	Moving Average	*****	*****	*****
	Probit	0,01	0,00	0,14
	Logit	0,01	0,00	0,14
EETPS	Binomial	*****	*****	*****
	Moving Average	*****	*****	*****
	Probit	0,65	0,00	1025,03
	Logit	1,17	0,00	∞
EEHPS	Binomial	0,01	0,00	0,10
	Moving Average	0,01	0,00	0,04
	Probit	0,01	0,00	0,07
	Logit	0,01	0,00	0,07

LC_{50}: Mean Lethal Concentration; EITPS: Isopropanol extract of *P. stelis* stem; EIHPS: Isopropanol extract of *P. stelis* leaves; EETPS: Ethanol extract of *P. stelis* stem; EEHPS: Ethanol extract of *P. stelis* leaves; ***** Data not provided by the program; ∞: infinite.

Table 2. Mean lethal concentration of ethanol and isopropanol extracts of *F. pumila* on *Artemia* sp. nauplii

Extracts	Method	LC_{50} (µg/mL)	Confidence limit (95%) Lower	Upper
EETFP	Binomial	0,66	0,01	1000,00
	Moving Average	1,20	0,15	5,75
	Probit	0,72	0,00	24,62
	Logit	0,75	0,00	∞
EEFFP	Binomial	*****	*****	*****
	Moving Average	0.44	0,07	1,80
	Probit	0,16	0,00	13,03
	Logit	0,19	0,00	∞
EEHFP	Binomial	3162,28	0,00	0,10
	Moving Average	3162,28	0,00	∞
	Probit	1615,74	144,94	∞
	Logit	2330,09	0,00	∞
EIFFP	Binomial	1,77	0,00	10000,00
	Moving Average	0,98	0,00	∞
	Probit	0,62	0,04	3,80
	Logit	0,63	0,01	6,66
EITFP	Binomial	2463,79	1000	10000,00
	Moving Average	*****	*****	*****
	Probit	2859,33	0,00	∞
	Logit	282830,10	0,00	∞
EIHFP	Binomial	11,91	1,00	10000,00
	Moving Average	85,88	27,32	345,37
	Probit	130,78	0,00	∞
	Logit	526,38	0,00	∞

LC_{50}: Mean Lethal Concentration: EETFP: Ethanol extract of stem of *F. pumila*; EEFFP: Ethanol extract of fruit of *F. pumila*; EEHFP: Ethanol extract of leaves of *F. pumila*; EIFFP: Isopropanol extract of fruit of *F. pumila*; EITFP: Ethanol extract of stem of *F. pumila*; EIHFP: Ethanol extract of leaves of *F. pumila;* *****: Data not provided by the program; ∞: infinite.

Larvicidal activity: The ethanol and propanol leaf extracts from *F. pumila* revealed no toxicity against *A. aegypti* larvae. In the case of *P. stelis* leaf, although the mean lethal concentration of ethanol and isopropanol extracts were 9726,94 and 556,112μg/mL, respectively, these findings did not indicate significant toxicity levels.

DISCUSSION

Our analysis of Isopropanol and ethanol extracts from *F. pumila* and *P. stelis* revealed specific compounds with distinct chemical compositions. *F. pumila* exhibited a rich presence of flavonoids, tannins, and polyphenols, while *P. stelis* showcased a diverse range of tannins, polyphenols, triterpenes, anthraquinones, and flavonoids. These results align with prior studies by Martins et al. (2006), Leong et al. (2008), Noronha et al. (2014), and Xiao et al. (2022), who reported the detection of steroid sterols, flavonoids, tannins, and phenolic compounds, respectively. Additionally, Oliveira et al. (2009), Sirisha et al. (2010), and Kaur (2012) documented the presence of triterpenes in their findings.

These compounds exert antimicrobial activity through enzymatic inhibition, protein interaction, and cell wall complex formation (Domingo & López, 2003). Flavonoids, which have phenolic hydroxyl groups, readily penetrate the cell membrane of microorganisms and bind to their proteins, leading to protein denaturation and mutagenic effect (Tereschuk, 2007). Flavonoids form a complex with the bacterial cell wall, causing the death of the microorganism (Goyal, 2012). Tannins can inactivate enzymes, protein transport, and microbial adhesion, thus rendering the microorganism inactive. The number of hydroxyl groups on the aromatic rings of polyphenols influences their antimicrobial potency (Domingo & López, 2003). Terpenes disrupt the lipid bilayer of cell membranes, causing intracellular leakage (Bueno-Sánchez et al., 2009). Certain triterpenes have been reported as inducers of cancer-cell apoptosis, establishing these compounds as harbingers for the prevention and treatment of cancer (Chudzik et al., 2015).

Despite the well-established antibacterial properties of several *Ficus* species, such as F. *microcarpa* (Ragasa et al., 1999; Sirisha et al., 2010); *F. craterostoma*, *F. cyathistipula*, *F. drupacea*, *F. hispid a*, *F. macrophylla*, *F. mucuso*, and *F. villosa* (Tkachenko et al., 2016), research on *F. pumila* in this regard remains notably scarce.

Our investigation revealed no discernible antifungal properties in the extracts of *F. pumila* and *P. stelis* against the array of fungal strains examined, which contrasts with the findings of Noronha et al. (2014), who demonstrated that ethanol extracts of *F. pumila* exhibited antifungal efficacy against the yeasts *C. albicans* and *S. cerevisiae*. Other studies have also documented the antifungal activity of several *Ficus* species, including *F. carica*, against various fungi: *Fusarium oxysporum* and *A. niger* (Rashid et al., 2014), *Candida famata* (Al-Askari et al., 2013) and *Trichoderma viride* (Yan et al. 2011).

The absence of antifungal activity in our study may be attributed to factors such as resistance to fungicides or lack of antifungal properties in the chemical composition of the spices under investigation, as proposed by Joklik (1995), who set forth numerous defense mechanisms employed by fungi, such as diminished drug permeability, enzymatic inactivation of inhibitors, modification of drug-receptor sites, and heightened synthesis of metabolites antagonistic to the drug.

Our study, which may well be the first report of positive toxicity against *Artemia* sp., identified the propanol extracts of the stem and leaves of *P. stelis* and the ethanol extract of the fruit of *F. pumila* as the most cytotoxic against *Artemia* sp.

Artemia testing, described by Olmedo et al. (2023), is a simple and cost-effective method for detecting cytotoxic drugs with specific mechanisms of action, such as protein synthesis inhibitors, mitotic inhibitors, DNA topoisomerase I inhibitors, and agents that interfere with the caspase cascade.

Our investigation into the ethanol and isopropanol extracts of *F. pumila* and *P. stelis* found no noteworthy toxic effects on *A. aegypti* larvae. As far as we know, there have been no documented instances of larvicidal activity from these extracts against *A. aegypti*. It is worth noting, however, that recent literature has documented promising larvicidal activity against *Anopheles stephensi* larvae (Rodrigues et al., 2023) and other mosquito species using titanium dioxide nanoparticles synthesized from the aqueous leaf extract of *Ficus religiosa* (Soni & Dhiman, 2020; Murugesu et al., 2021), also belonging to the Moraceae family.

Exploring plant species to uncover novel bioactive compounds in natural products from plants is an ambitious undertaking and an area of extensive research. We are committed to unwavering dedication in our pursuit of chemical compounds with inhibitory effects against microorganisms. We encourage efforts to continue to standardize extraction and elucidation methods focused on the analysis and characterization of the molecular structure of chemical compounds, using chromatography and spectroscopy. Furthermore, as Carrillo and Galván (2022) acknowledge, in vitro assays will enhance the effectiveness of research on various compounds with inhibitory effects on microorganisms. In addition, although the exact determination of those compounds may not be readily conclusive, we must keep researching, always warding off the potentially harmful effects and toxicity they may cause.

ACKNOWLEDGEMENTS

We thank Instituto Nacional de Investigaciones Agrícolas (INIA), Sucre State, Venezuela, and the Universidad de Oriente, Sucre, Venezuela, for the technical contributions in the preparation of this research.

ETHICAL, CONFLICT OF INTEREST AND FINANCIAL STATEMENTS

The authors declare that they have fully complied with all pertinent ethical and legal requirements, both during the study and production of the manuscript; that there are no conflicts of interest of any kind; that all financial sources have been fully and clearly stated in the Acknowledgment section; and that they fully agree with the final edited version of the article. A signed document has been filed in the journal archives.

The authors' contribution to the manuscript are as follows: VL: Scientific director, study design and preparation of the manuscript. MM: sample processing and biological activity testing. LR: Sample processing and biological activity testing, VH: Laboratory manager and data analysis. All co-authors: Manuscript preparation and final approval.

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APPENDIX

The plants were collected in the year 2019.

In natural products, the number of organisms is not significant; the mass of the extracts is what matters. Below are the masses and yields of the plant organs.

Masses and percentage yields of crude extracts in isopropanol of *F. pumila* and *P. stelis.*

Plant Organ	Masses fresh (g)	Masses of extracts (g)	Percentage yields (%)
Stems *P. stelis*	30,2	16,2	53,6
Leaves *P. stelis*	40,7	33,8	83,0
Leaves *F. pumila*	122,1	16,1	13,2
Stems *F. pumila*	115,8	20,2	17,4
Fruits *F. pumila*	351,0	40,4	11,5

g: grams s, %: yield percentage.

Masses and percentages of yield of crude ethanol extracts of *F. pumila* and *P. stelis*.

Órgano vegetal	Masses fresh (g)	Masses of extracts (g)	Percentage yields (%)
Stem *P. stelis*	41,0	22,3	54,3
Leaves *P. stelis*	39,9	21,3	53,4
Leaves *F. pumila*	84,9	12,0	14,1
Stems *F. pumila*	86,9	18,5	21,2
Fruits *F. pumila*	212,0	13,2	6,2

g: grams s, %: yield percentage.