



## Fungal pathogens of *Thymus vulgaris* (Thyme): Nutrient and anti-nutrient compositions

Wekhe E O, Uwaebute R N, Chuku E C, Chukwu C S

Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Rivers State, Nigeria

### Abstract

Research on fungal pathogens of *Thymus vulgaris* (Thyme): nutrient and anti-nutrient compositions were carried out in the Department of Plant Science and Biotechnology, Rivers State University. Leaves of thyme were purchased from fruit garden market in Port Harcourt. Mycological studies were carried out in Plant Pathology laboratory while nutrient and antinutrient compositions were done in Food Science and Technology in the Rivers State University. The mycological result revealed the presence of three microorganisms which are *Rhizoctonia spp*, *Fusarium solani* and *Alternaria alternata* with percentage incidences of 19, 15 and 4% respectively. Proximate composition result revealed Lipid (30.16±0.05%) to be highest and Ash (2.30±0.00%) was the least. For minerals, potassium was highest (372.67±28.29%) and iron (4.70±0.17%) the least. Phytochemical results revealed glycoside, oxalates, saponin, tannin, carotenoid, flavonoid, polyphenols and lignant. In conclusion, thyme is rich in nutrients but prone to fungal contamination. Infected leaves should not be consumed as it can lead to the buildup of fungal load in the body, causing grave consequences.

**Keywords:** Fungal pathogens, nutrient and antinutrient compositions, thyme

### Introduction

*Thymus vulgaris* (Thyme) is a flowering plant that belongs to the Lamiaceae family native to Southern Europe, and is distributed worldwide (Hosseinzadeh *et al.*, 2015) [15]. In Africa, the plant has been domesticated in Egypt, Morocco, Algeria, Tunisia, Libya (Stahl-Biskup and Sáez, 2002), Cameroon (Nkouaya *et al.*, 2007), Nigeria (Kayode and Ogunleye, 2008) [17], and South Africa (Schmitz, 2015). Thyme has been used for decades as a flavoring agent, culinary herb, and herbal medicine (Stahl-Biskup and Venskutonis, 2012) [32]. According to Ekoh *et al.*, (2014), the plant as an infusion is used to treat cough, diabetes, cold and chest infections; and in syrup form for digestive upset. They also reported its ability to soothe sore throat, antiseptic, antibiotic, and antifungal properties.

Reddy *et al.*, (2014) [26], reported its active ingredient, thymol, being active against enterobacteria and cocci bacteria. Zekovic *et al.*, (2003) [40] and Khajeh (2011) [18] also reported the presence of terpenoids such as carvacrol, menthol, camphor and phytol to be present in lower amounts in the thymus extracts. On like every other plants, Thyme is also prone to diseases. Reddy *et al.*, (2014) [26], reported the diseases that affect thyme most often to be root rot (*Rhizoctonia*), grey mold (*Botrytis cinerea*), and Alternaria blight (*Alternaria brassicicola*) diseases which can spread through the soil, water, air, infected tools, animals, and even gardeners themselves.

### Materials and Methods

#### Collection of Sample

Samples were collected from fruit garden, Port Harcourt, Nigeria. A portion of the sample was taken to the Department of Food Science and Technology for nutrient and anti nutrient analysis and the other portion reserved for fungal isolation at Plant Pathology laboratory Rivers State University.

#### Sterilization of Glass wares for Fungi Isolates

All the glass wares were first soaked and washed thoroughly with tap water and detergent solution and then mixed with several changes of distilled water in order to completely remove traces of detergent. They were Air dried completely before sterilizing them in steam oven at temperature 121 degrees Celsius for 15 minutes and then allowed to cool down at room temperature before usage. The entire working surface was also disinfected with ethanol to reduce contamination. This was done according to the method of Wekhe *et al.*, (2022) [37].

#### Isolation, Subculturing and Identification of Pathogens

Isolation of the fungi was carried out as described by Chuku (2009) [9]. The infected thyme plants showing disease symptoms were first washed and surface sterilized with 10% ethanol using a cotton wool. Four small pieces from the margin of lesion of each sample were directly inoculated aseptically on prepared plates of Sabaraud Dextrose Agar (SDA) and Incorporated at 28°C for 3 to 5 days. When fungal growth from the tissue was visible, fungi were subcultured onto SDA to obtain pure cultures for identification. The pure cultures were safely refrigerated at 4°C to prevent any fungal growth in the plates. Identification of microorganisms was based on growth patterns, colour of mycellia and microscopic examination of vegetative and reproductive structures. For each microscopic examination a streak of fungal mycelium was placed on a clean glass slide. One drop of cotton blue lactophenol was added and the cover slip placed. The slide was mounted on the microscope and observed at magnification of X10 and X40. Morphological characteristics of fungi isolated were determined and identified with the aid of identification guide (Giraldo and Crous, 2019) [13]

#### Proximate Composition of *Thymus vulgaris*

The first sample was analyzed for moisture, Ash, lipid, fibre, carbohydrate and protein (Kjeldahl methods),

proximate analysis was determined according to the method of Association of Analytical Chemistry (AOAC, 2005) [2].

### Determination of mineral contents

Iron Content was done according to the method of Achikanu and Eze-Steven (2013) [4]. Phosphorous content was according to the method of Liu *et al.*, (2017). Determination of Vitamin A, C and Thiamin was AOAC (2005) [2] method.

### Determination of Anti nutrient

Oxalate content was determined by the method of Day and Underwood (1986)

## Results

### Proximate composition of *Thymus vulgaris*

**Table 4.1:** Proximate composition of *Thymus vulgaris* (%)

Parameter	Composition
Moisture	12.65±0.00
Ash	2.30±0.00
Lipid	30.16±0.05
Fibre	2.46±0.11
Carbohydrate	25.28±1.35
Protein	24.40±1.03

**Table 4.2:** Mineral compositions of *Thymus vulgaris* (mg/100g)

Parameter	Composition
Calcium	44.67±0.57
Iron	4.70±0.17
Magnesium	116.67±0.57
Phosphorous	116.67±1.52
Potassium	372.67±28.29
Sodium	25.93±0.80

**Table 4.3:** Vitamins Composition of *Thymus vulgaris* (mg/100g)

Vitamin C	2.40±0.10
Vitamin A	0.04±0.00
Thiamin	0.04±0.00
Chlorophile	0.06±0.00

**Table 4.4:** Phytochemical Composition of *Thymus vulgaris* (%)

Parameter	Composition
Glycoside	0.07±0.00
Oxalate	0.03±0.00
Saponin	0.07±0.00
Tannin	0.02±0.00
Carotenoid	25.10±0.95
Polyphenol	6.29±0.00
Flavonoid	2.93±0.06
Lignant	1.96±0.00

**Table 4.5:** Fungi Isolate

Fungi Isolates	Macroscopic Examination	Microscopic Examination	Probable Organism	% Incidence
Isolate 1	On PDA, the colony was white, later cream yellow, with low growth of cottony aerial mycelium	Non- septate, broad and unbranched hyphae, brown in colour, solitary sporangiophores, sporangia are globose with a flattened base.	<i>Rhizoctonia solani</i>	19%
	Fast growing colours, up to 4.5cm in 4days on PDA, aerial mycelium bluish brown when sporodocia are present	Micronodia were formed after 3-7 days form short multiple branched conidiophores, fusiform, cylindrical to val, one to two celled and formed long lateral phialides	<i>Fusarium oxysporum</i>	15%
Isolate 3	Fast growing with irregular margin, grey/olivaceous to black, wholly colony with black reversed side.	Septate, conidiophores mostly unbranched or single with large conidia	<i>Alternaria alternata</i>	4%

## Discussion

### Proximate composition of thyme

Table 4.1 presents the proximate composition of thyme as shown in Table 4.1, the moisture content recorded 12.65±0.00, Ash (2.30±0.00), Lipid (30.16±0.05), Fibre (2.46±0.11), Carbohydrate (25.28±1.35), and Protein (2.46±0.11). The moisture content of this study is higher than 4.59% and 4.03 % for thyme and Basil reported by Hammam *et al.*,(2025) [14] but lower than *Moringa oleifera* leaves (76.53%) and *Corchorus oiltoorius* (79.98%) reported by Sadowska *et al.*, (2017) [28], *Heinsia crinita* (26.4±0.1), and *Lasianthera africana* (65.45±0.07) reported by Wekhe *et al.*, (2022) [37]. Low moisture content facilitates storage stability. The Ash content of this study is higher than 1.5g/100% of fresh *Telfaria occidentalis* reported by Orhuamen *et al.*, (2012) [25] according to Fagbohun *et al.*, (2012) [12], Ash content in leafy vegetables is a reflection of the amount of mineral element present in the vegetables. The Lipid content of this study is lower than *Gnetum africanum* (37.80%) reported Ndomou *et al.*, (2014) [21] and higher than *Heinsia crinita* (15.2±0.01%), and *Lasianthera africana* leaves (3.51±0.02) reported by Wekhe *et al.*, (2022) [37]. Plant lipid act as a signal molecule to regulate cell metabolism and are also essential for the integrity of cells an organelles by acting as a hydrophobic barrier for the membrane as reported by Ohlrogge and Browse (2013) [24].

The carbohydrate content of thyme aligns with 22.91-45.03% of Hamman *et al.*, (2025). Kassegn (2016) [16] reported a utilizable carbohydrate value of approximately 55-60%. The protein content 24.40±1.03% in this study is higher 9.6-11.1% of thyme aerial parts reported by Kassegn (2016) [16] but exceeds other herbs 5.61- 13.57% but matches optimized spring harvest samples (Banday *et al.*,2025, Roy *et al.*, 2025) [5, 27].

### Mineral composition of thyme

The mineral analysis of thyme in Table 4.2 revealed potassium as the predominant element (372.67 ± 28.29 mg/100 g), followed by magnesium and phosphorus (116.67 ± 0.57 and 116.67 ± 1.52 mg/100 g, respectively), calcium (44.67 ±0.57), sodium (25.93±0.80) and iron (4.70±0.17) least. The calcium content of this study falls below *Hua. Gabonii* fruit (74mg/100g), but higher than *M. whited* (106mg/100g), reported by McDowell (2003) [19]. The iron content of this study is low compared to *Echinops giganteu* (206.4mg/100g) reported by (Vineetha, 2014). Iron helps in growth and development, and also in the production of hemoglobin and myoglobin, Bhat *et al.*, (2010) [7]. The magnesium and phosphorus content of this study were high and in line with Kessegn (2016). The potassium content was high (372.67±28.29mg/100g), and in line with Bouba *et al.*, (2012) [8] and Biel *et al.*,

(2023) [6]. The high potassium content reflects the physiological importance of this element in osmotic regulation, stomatal function and enzymatic activation in the plant. Sodium content is low, low sodium levels have also been reported by Waheed *et al.*, (2014) [34], who emphasized thymes suitability for use in low sodium- salt blends and functional foods.

#### Vitamin Composition of thyme

The vitamin content of thyme in Table 4.3 revealed vitamin A ( $0.04 \pm 0.00$ ), vitamin C ( $2.40 \pm 0.10$ ) and thiamine ( $0.06 \pm 0.00$  mg/100g). These records are low compared to findings of Vineetha, (2014) who recorded vitamin A (4751IU) and vitamin C (160.1mg) and thiamine as (0.48g).

#### Phytochemical composition of thyme

The phytochemical analysis demonstrated that *T. vulgaris* is particularly rich in carotenoids ( $25.10 \pm 0.95$  mg/100 g), polyphenols ( $6.29 \pm 0.00$  mg/100 g), and flavonoids ( $2.93 \pm 0.06$  mg/100 g). These compounds are widely associated with antioxidant, anti-inflammatory, and antimicrobial activities. Carotenoids contribute to provitamin A activity and play a protective role against oxidative stress-related chronic diseases, including cardiovascular disorders and certain cancers (Meléndez-Martínez *et al.*, 2021) [20]. Polyphenols and flavonoids are key contributors to thyme's bioactivity, with recent investigations linking these compounds to strong radical-scavenging capacity and modulation of inflammatory pathways. The values obtained in this study align with contemporary reports identifying thyme as a phenolic-rich herb, with compounds such as rosmarinic acid, luteolin, and apigenin derivatives playing central roles in its biological activity (Nieto, 2020; Shan *et al.*, 2022) [22, 30].

The presence of saponins and glycosides, though in small quantities, is noteworthy due to their reported antimicrobial, hypocholesterolemic, and immunomodulatory effects. Tannins were detected at low levels ( $0.02 \pm 0.00$  mg/100 g), suggesting minimal antinutritional impact while still contributing to antimicrobial and antioxidant properties. Oxalate content was also low ( $0.03 \pm 0.00$  mg/100 g), indicating a negligible risk of mineral chelation or reduced bioavailability. Lignans ( $1.96 \pm 0.00$  mg/100 g) further enhance the phytochemical profile, as these compounds have been associated with cardioprotective and antioxidant effects in plant-based diets.

Collectively, the proximate, mineral, vitamin, and phytochemical compositions observed in this study substantiate the classification of *Thymus vulgaris* as a nutritionally valuable and pharmacologically relevant herb. The results align with current research trends emphasizing the role of culinary herbs as sources of bioactive compounds capable of contributing to disease prevention, food preservation, and functional food development. Variations between the present findings and previously published data may be attributed to differences in geographical origin, agronomic conditions, maturity stage, and post-harvest processing.

#### Fungal isolates found in *Thymus vulgaris*

The three fungal isolates recorded in Table 4.5 were identified morphologically and microscopically as *Rhizoctonia solani*, *Fusarium oxysporum*, and *Alternaria alternata*, with incidence values of 19%, 15%, and 4%,

respectively. *R. solani* was the most frequently isolated fungus. According to Yi *et al.*, (2025) [39], *R. solani* is a globally distributed soil pathogen implicated in damping off and root rot across diverse crops due to its saprophytic survival structures and broad host range. Abdullahi *et al.*, (2025) [3] also identified *R. solani* in recent surveys of soil-borne pathogens where it often surpasses other fungi in detection frequency. *F. oxysporum* have been reported in researches as common and aggressive soil-borne fungus frequently isolated from irrigated soils and vegetable crops where it causes vascular wilt and root diseases (Chuku *et al.*, 2009, Chuku, *et al.*, 2018, Obire *et al.*, 2016, Wekhe *et al.*, 2020, Wekhe *et al.*, 2021) [9, 10, 23, 35]. *A. alternata* was the least common isolate with 4% incidence reflecting its ecological niche as a saprophyte or weak pathogen under certain environmental conditions. The ranking of the isolates in this study aligns with data from studies on wheat and vegetable roots which reported *R. solani* and *Fusarium species* at higher appearance and frequency rates compared to other fungi in soil and root samples (Abass *et al.*, 2021) [1]. Moreover, technological advances such as hyperspectral imaging combined with machine learning have been applied to detect *A. alternata* in plant tissues alongside *F. oxysporum*, highlighting its diagnostic relevance despite lower prevalence (Seyed *et al.*, 2024) [29]. In conclusion, this research suggests that thyme, either in its fresh or essential oil form is rich in essential plant nutrients known to possess antimicrobial, antioxidative, anti-inflammatory, improves digestion and immunity. These health properties are the reasons why the consumption of this potent herb is advocated. However, due to the fact that this important herb is prone to numerous fungal contaminations, care must be taken during its preservation to reduce the fungal loads, which when consumed could lead to unpleasant health challenges.

#### References

1. Abass MH, Madhi QH, Matrood AAA. Identity and prevalence of wheat damping off fungal pathogens in different fields in Basrah and Maysan provinces. Bulletin of National Research centre, 2021, 45-51, 1-12. <https://doi.org/10.1186/s42269-021-00506-0>.
2. AOAC. Official methods of analysis. 18th edition. Association of Office Analytical Chemists, Washington, D.C., 2005.
3. Abdullahi M, Chimbekujwo IB, Bristone B, Anoruo-D CA, Yakubu NL, Abdullahi MM, *et al.* Survey of soil-borne pathogenic fungi affecting some vegetables in Gerio irrigation farm, Adamawa Nigeria. International Journal of Research and Scientific innovation (IJRSI), 2025;12(1):553-562.
4. Achikanu CE, Eze-Stephen PE, Ude CM, Ugwuokolie OC. Determination of the vitamin and mineral composition of common leafy vegetables in South eastern Nigeria. International Journal Curriculum Microbiology Applied Science, 2013;2(11):347-353.
5. Banday MT, Wani M, Alqahtani FM, Adriani L, Alhomrani M, Adil S, *et al.* Enhancing meat-type chicken performance through *Thymus vulgaris* leaf powder supplementation by affecting serum lipid profile, stress physiology, immunity, antioxidants, cecal microbiology, and jejunal histomorphology. Archives of Animal Breeding, 2025;68:311-323. <https://doi.org/10.5194/aab-68-311-2025>.

6. Biel W, Pomietio U, Witkowicz R, Piattkowska E, Kopec A. Proximate composition and Antioxidant activity of selected morphological parts of herbs. *Applied Sciences*,2003;13(3):1413.
7. Bhat LD, Eagle AK, Magnus E, Hirsch TA, Elisabeth GS. Comparative determinants of 4 year cardiovascular event rates in stable outpatients at risk of or with atherothrombosis. *Jama*,2010;304(12):1350-1357.
8. Bouba AA, Yanou NNN, Foyet H, Didi SJ, Moses C, Mbofungti. Proximate composition, mineral and vitamin content of some wild plants used as spices in Cameroon. *Food and Nutrition Sciences*,2012;3:423-432.
9. Chuku EC. Fungi responsible for the spoilage of Plantain (*Musa paradisiaca*) at various ripening stages. *Acta Agronómica Nigeriana*,2009;9(1&2):35-40.
10. Chuku EC, Chuku OS, Ajuru MG. Studies on the propagation, phytochemical properties, storage utilization and shelf- life of *Asystasia gangetica*. *Journal of Agriculture and Veterinary science*,2018;11(8):63-67.
11. Dauqan EM, Abdullah A. Medicinal and functional values of thyme (*Thymus vulgaris* L.) herb. *Journal of Applied Biology & Biotechnology*,2017;(02):017-22.
12. Fagbohun ED, Lawal OU, Ore ME. The proximate mineral and phytochemical analysis of the leaves of a *Occimum gratissimum* L. *Melanthera scandens* A., and *Leea guineensis* L. and medicinal value. *International Journal of applied biology and pharmaceutical technology*,2012;3:15-22.
13. Giraldo A, Crous PW. Inside Plectosphaerellaceae. *Studies in Mycology*,2019;92(1):227-286.
14. Hammam MA, El-Sayed SM, Ebrahim AA. Biochemical studies on thyme and basil essential oils. *Menoufia Journal of Agricultural Biotechnology*,2025;10(1):21–30.
15. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of *Thymus vulgaris*. *International Journal of Clinical Medicine*,2015;6:635–642. doi: 10.4236/ijcm.2015.69084.
16. Kassegn HH. Determination of the proximate composition of the wild Abyssinian thyme herb (*Thyme schimperii* L.) grown in highlands of Southern Tgray, North Ethiopia. *Food Science and Quality Management*,2016;50:1-3.
17. Kayode J, Ogunleye TO. Checklist and Status of Plant Species Used as Spices in Kaduna State of Nigeria. *Research Journal of Botany*,2008;3:35-40. <https://doi.org/10.3923/rjb.35.40>.
18. Khajeh M. Optimization of process variables for essential oil components from *Satureza hortensis* by supercritical fluid extraction using Box-Behnken experimental design. *The Journal of supercritical fluids*,2011;55(3):944-948.
19. McDowell WH. Dissolved organic matter in soils- future directions and unanswered questions. *Geoderma*,2003;113(3-4):179-186.
20. Meléndez-Martínez AJ, Böhm V, Borge GIA, Cano MP, Fikselová M, Gruskiene R, *et al.* Carotenoids: Considerations for their analysis, regulation, and health effects. *Journal of Agricultural and Food Chemistry*,2021;69(7):1999–2014. <https://doi.org/10.1021/acs.jafc.0c06629>.
21. Ndomou M, Mezajoug KLB, Tchiegang C. Physicochemical properties of leaves of *Gnetum africanum* (L.) and *Gnetum bucholzianum* (L.) (Gnetaceae) from Cameroon. *American Journal of Research Communication*,2014;2(12):101-112.
22. Nieto G. A review on applications and uses of *Thymus* in the food industry. *Plants*,2020;9(8):961. <https://doi.org/10.3390/plants9080961>.
23. Obire O, Wekhe EO, Steve A. Microorganism associated Microorganism associated with post harvest diseases of yam (*Dioscorea* species) in Barns. *Current studies in Comparative Education. Science and Technology*,2016;3(1):108-117.
24. Ohlrogge J, Browse J. Lipid biosynthesis. *Plant Cell*, 2013, (also cited as 1995;7:957–970).
25. Orhuamen EO, Olorunmaiye KS, Adeyemi CO. proximate analysis of fresh and dry leaves of *Telferia occidentalis* (Hook. F) and *Talinum triangulate* (Jacq.) wild. *Croatian journal of food technology biotechnology and nutrition*,2012;7(3-4):188-191.
26. Reddy P, RaviVital K, Varsha P, Satyam S. Review on *Thymus vulgaris* Traditional Uses and Pharmacological Properties. *Medicinal Aromatic Plants*,2014;3:2167-0412. doi: 10.4172/2167-0412.1000164.
27. Roy N, Sharma N, Mohite AM. Evaluating the impact of various drying processes on the quality attributes of thyme (*Thymus vulgaris* L.). *Fermentation*,2025;7(3):Article59. <https://doi.org/10.3390/fermentation7030059>.
28. Sadowska U, Kipek A, Kourimska L, Zarubova L, Kloucek P. The effects of drying methods on the concentration of compounds in sage and thyme. *Journal of Food Process Preservation*, 2017.
29. Seyed MJ, Ahmad B, Keyvan AV, Yiannis A, Kamran R. Early detection and Spectral signature identification of tomato fungal diseases (*Alternaria alternata*, *Alternaria solani*, *Botrytis cinerea* and *Fusarium oxysporum*) by RGB and hyperspectral imag analysis and machine learning. *Heliyon*,2024;10:e38017. <https://doi.org/10.1016/J.heliyon.2024.e38017>.
30. Shan B, Cai YZ, Brooks JD, Corke H. Antioxidant capacity of culinary herbs and spices. *Journal of Food Composition and Analysis*,2022;105:104–112. <https://doi.org/10.1016/j.jfca.2021.104112>.
31. Stahl-Biskup E, Sáez F. The History, Botany and Taxonomy of the Genus *Thymus*. *The Genus Thymus*. Taylor and Francis, Inc.; London, UK, 2022.
32. Stahl-Biskup E, Venskutonis RP. *Handbook of Herbs and Spices: Second Edition*,2012;1:499-525.
33. Vineetha. 22 Amazing benefits and uses of Thyme, 2017. Available from: <http://www.healthbeckon.com/thyme-benefits/>.
34. Waheed M, Aadil RM, Ahmad MI, Safdar W, Khalid S, Kausar R, *et al.* Phytochemical profiling and therapeutic potential of thyme (*Thymus* spp.): A medicinal herb. *Food Science & Nutrition*,2024;12(12):9893–9912. <https://doi.org/10.1002/fsn3.4563>.
35. Wekhe OE, Chuku EC, Nmom FW. Determination of nutrient composition and associated spoilage fungi of different varieties of breadfruit. *International Journal of*

- Research and Innovation in Applied Science,2020:5(9):1-4.
36. Wekhe OE, Chuku EC, Okogbule FNC. Effect of fungi flora in the nutrient quality of *Artocarpus camansi* (Breadnut). Journal of Agriculture, Environmental Resource and Management,2021:5(4):991-996.
  37. Wekhe EO, Chuku EC, Agbagwa SS, Brown OP. Nutrient composition of *Lasianthera africana* (Nkanka) and it's spoilage moulds. Journal of Biology and Genetic Research,2022:8(2):13-21.
  38. Yang J, Sun C, Zhang Y, Fu D, Xiaodong, Yu T. "Induced resistance in tomato fruit by  $\gamma$ -aminobutyric acid for the control of alternaria rot caused by *Alternaria alternata*". Food Chemistry,2017:221:1014–1020. doi: 10.1016/j.foodchem.2016.11.061.
  39. Yi RH, Zeng XM, Li KY. *Rhizoctonia solani* causes okra (*Abelmoschus esculentus*) seedling damping off in South China with biological characterization and fungicide sensitivity profiling. Sci Rep,2025:15:34156. <https://doi.org/10.1038/s41598-025-14836-w>.
  40. Zekovic ZP, Lepojevic ZD, Milosevic SG, Tolic AS. Modelling of the thyme: Liquid carbondioxide extraction system. APTEFF (Acta Periodica Technologica),2003:34:125-133.