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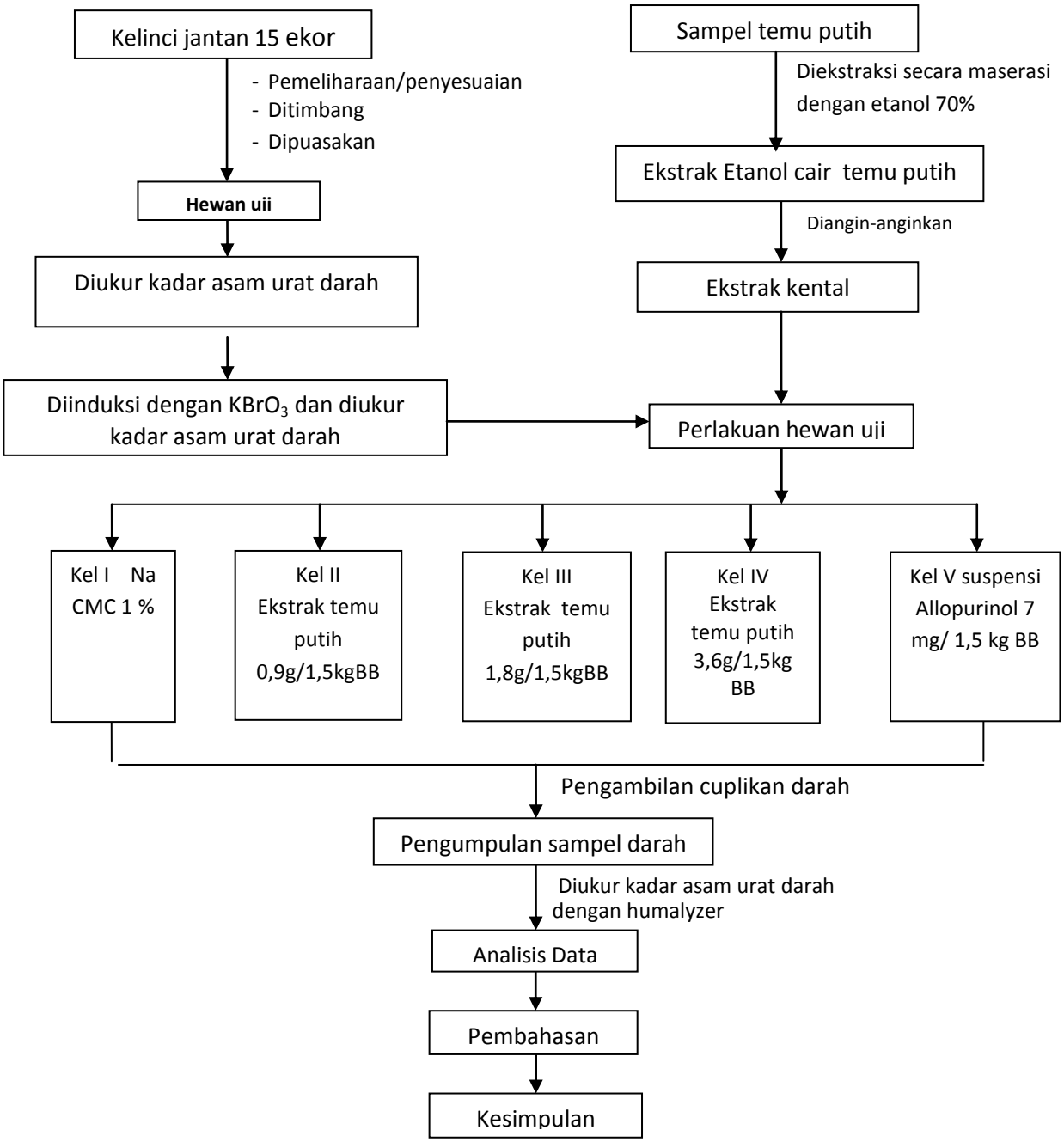
## LAMPIRAN I

## SKEMA KERJA PENELITIAN

## PENGARUH EKSTRAK RIMPANG TEMU PUTIH (*Curcuma zedoaria*) TERHADAP KADAR ASAM URAT PADA KELINCI (*Oryctolagus cuniculus*)



## LAMPIRAN II

## KONVERSI DAN PERHITUNGAN DOSIS ALLOPURINOL

### 1. Perhitungan Suspensi Allopurinol

Dosis allopurinol untuk manusia : 100 mg

Faktor konversi dosis manusia ke kelinci (BB 1,5 kg) : 0,07

Dosis untuk kelinci dengan BB 1,5 kg = 100 mg x 0,07

= 7 mg

Acuan volume pemberian maksimum peroral untuk kelinci dengan bobot badan 2,5 kg = 20 ml

Dosis untuk kelinci dengan bobot badan 2,5 kg = $\frac{2,5}{1,5}$ × 7 mg

= 11,6 mg

Sediaan stok yang dibuat = 100 ml

Dibutuhkan allopurinol = $\frac{11,6\ \text{mg}}{20\ \text{ml}}$ x 100ml

= 58 mg

### 2. Penimbangan Allopurinol

Akan dibuat sebanyak 100 ml suspensi allopurinol sehingga allopurinol yang dibutuhkan sebanyak = $\frac{11,6\ \text{mg}}{20\ \text{ml}}$ x 100 ml = 58 mg

Bobot 20 tablet = 8,8 g= 8800 mg

= 8800 mg/20 tablet

= 440 mg/tablet

Bobot yang ditimbang = (58 mg/100 mg) x 440 mg

=255,2mg (setara dengan 58 mg)

## LAMPIRAN III

## ANALISIS STATISTIK PENURUNAN KADAR ASAM URAT KELINCI YANG DIBERI PERLAKUAN DENGAN EKSTRAK ETANOL RIMPANG TEMU PUTIH (*Curcuma zedoaria*), YANG DIBANDINGKAN DENGAN KONTROL

| Perlakuan | Replikasi / Penurunan kadar asam urat (mg/dl) | | | Jumlah | Rata-rata |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | | |
| Kontrol negatif (NaCMC) | 0,4 | 0,5 | 0,3 | 1,2 | 0,40 ± 0,1 |
| Ekstrak Etanol 0,9 g | 0,9 | 0,5 | 1,1 | 2,5 | 0,83 ± 0,3 |
| Ekstrak Etanol 1,8 g | 1,2 | 1,1 | 1,1 | 3,4 | 1,13 ± 0,05 |
| Ekstrak Etanol 3,6 g | 1,6 | 1,1 | 1,4 | 4,1 | 1,36 ± 0,25 |
| Kontrol positif (Allopurinol) | 1,0 | 1,2 | 1,1 | 3,3 | 1,10 ± 0,1 |
| Jumlah | 5,1 | 4,4 | 5,0 | 14,5 | |
| Rata-rata total | | | | | 0,96 |

Tabel Analisis varian

| **Sumber Keragaman** | **Derajat Bebas** | **Jumlah Kuadrat** | **Kuadrat Tengah** | **F Hitung (Fh)** | **F Tabel (Ft)** | | **Ket** |
|---|---|---|---|---|---|---|---|
| | | | | | **5 %** | **1 %** | ss |
| **Perlakuan** | 4 | 1,634 | 0,409 | 11,36 | 3,48 | 5,99 | |
| **Galat** | 10 | 0,360 | 0,036 | | | | |
| **Total** | 14 | 1,994 | | | | | |

Kesimpulan :

4. Perbedaan dosis perlakuan memberikan efek yang sangat berbeda terhadap penurunan kadar asam urat kelinci.
5. Dosis antara 0,9-3,6 g/1,5 kgBB memberinkan efek menurunkan kadar asam urat yang sangat nyata dibandingkan kontrol negatif.
6. Dosis 3,6 g/1,5 kgBB memiliki efek yang jauh lebih besar daripada kontrol positif sehingga perlu dipertimbangkan kemungkinan toksisitasnya.

## LAMPIRAN IV

## GAMBAR-GAMBAR PELAKSANAAN DAN HASIL PENELITIAN

Gambar 7. Temu Putih (*Curcuma zedoaria*) dan Rimpangnya


Gambar 9. Alat Humalyzer dan sentrifuge

Gambar 10. Serum Kelinci

**LAMPIRAN V**

**LABORATORIUM FARMAKOGNOSI-FITOKIMIA**
**FAKULTAS FARMASI**
**UNIVERSITAS HASANUDDIN**
*Kampus UNHAS Tamalanrea, Jl. Perintis Kemerdekaan Km.10*
*Telp. (0411) 588556, 586200, ext. 1093, Fax (0411) 590663, Makassar 90245*

## DETERMINASI TUMBUHAN

Determinasi tumbuhan dilakukan di Laboratorium Farmakognosi Fakultas Farmasi Universitas Hasanuddin dengan berpedoman pada Buku Flora of Java (Backer, C.A., and van den Brink, R.C.B., 1963). Hasil Determinasi tumbuhan sebagai berikut :

Suku : 207. Zingiberaceae
1a., 2b., 6b., 7a..(12. Curcuma L.)
Marga : 12. Curcuma
Jenis : 1b., 4b., 6a...(*Curcuma zedoaria* (Berg). Roscoe

Berdasarkan hasil determinasi tersebut maka diperoleh kepastian bahwa tumbuhan yang dideterminasi dan akan digunakan dalam penelitian ini adalah : **(*Curcuma zedoaria* (Berg). Roscoe**

Demikian keterangan determinasi ini diberikan untuk dipergunakan.

Makassar, 29 Desember 2010
Kepala Lab. Farmakognosi-Fitokimia
Fak. Farmasi Unhas

Prof. Dr. Gemini Alam, M.Si., Apt.
Nip. 19641231 199002 1 005

## LAMPIRAN VI

# Fluitest® UA

**URIC ACID**

analyticon

### Order information:

| Catalog No. | Contents | | | | | |
|---|---|---|---|---|---|---|
| 4848 | R1 | 8 x | 20 ml | R4 | 1 x | 5 ml |
| 4841 | R1 | 4 x | 100 ml | R4 | 1 x | 5 ml |

### Intended use:

Enzymatic in vitro test for the quantitative determination of uric acid in human serum and plasma.

### Summary:

Uric acid is the final product of purine metabolism in the human organism. Uric acid measurements are used in the diagnosis and treatment of numerous renal and metabolic disorders, including renal failure, gout, leukaemia, psoriasis, starvation or other wasting conditions, and of patients receiving cytotoxic drugs.
The oxidation of uric acid provides for two approaches to the quantitative determination of this purine metabolite. One approach is the reduction of phosphotungstic acid in an alkaline solution to tungsten blue, which is measured photometrically. The method is, however, subject to interferences from drugs and reducing substances other than uric acid.
A second approach, described by Praetorius and Poulson, utilizes the enzyme uricase to oxidise uric acid; this method eliminates the interferences intrinsic to chemical oxidation. Uricase can be employed in methods that involve the UV measurement of the consumption of uric acid or in combination with other enzyme to provide a colorimetric method.
The assay described here is a slight modification of the colorimetric method. The modifications were described by Siedel. This reaction, the peroxide reacts in the presence of peroxidase, DHBSA and aminoantipyrine to form a quinoneimine dye. The intensity of the red color is proportional to the uric acid concentration and is determined photometrically.

### Test principle:

Colorimetric endpoint assay (Uricase-PAP-method)

$$\text{Uric acid} + 2H_2O + O_2 \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

Uricase cleaves uric acid to form allantoin and hydrogen peroxide.

$$2H_2O_2 + H^+ + \text{DHBSA} + \text{4-Aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinone-diimine dye} + 4H_2O$$

The increase in absorbance is measured.

### Reagent concentration:

**R1:**

| | |
|---|---|
| Phosphate buffer pH 7.4 | 50 mmol/l |
| DHBSA* | 4 mmol/l |
| Uricase | 60 U/l |
| POD | 660 U/l |
| 4-Aminoantipyrine | 1 mmol/l |
| Preservative | |

*3,5-Dichloro-2-hydroxy-benzenesulfonic acid

**R4:**

Uric acid 6 mg/dl (356.9 µmol/l)

### Preparation and stability:

R1: ready for use
R4: ready for use
The reagents are stable up to the expiry date in the label when stored light protected at +2°C to +8°C.

| After opening: | | |
|---|---|---|
| | to expiry date | at +2°C to +8°C, protect from light |
| | 21 days | at +20°C to +25°C, protect from light |

Coloration of the reagent (reagent blank at 546 nm, 1 cm > 0.2) indicates a contamination, damage during transport or due storage at higher temperatures.

### Specimen:

*Serum/plasma*
Collect serum using standard sampling tubes.
Heparin, or EDTA-plasma

| Stability: | | |
|---|---|---|
| | 5 days | at +2°C to +8°C |
| | 6 months | at –20°C |

*Urine*
Collect urine without using preservatives.
Stability: Assay urinary uric acid as soon as possible. Do not refrigerate.
Centrifuge samples containing precipitate before performing the assay.

### Notes:

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.

### Limitations - interference:

Criterion: Recovery within ±10% of initial values
Icterus: No significant interference up to an index I of 12 (approximate conjugated and unconjugated bilirubin concentration 12 mg/dl)
Hemolysis: No significant interference up to an index H of 50 (approximate hemoglobin concentration: 50 mg/dl).
Lipemia (Intralipid): Triglycerides may interfere. There is poor correlation between turbidity and triglycerides concentration.
Elevated levels of ascorbic acid produces false low values.
Uricase reacts specifically with uric acid. Other purine derivatives can inhibit the uric acid reaction.

### Testing procedure:

**Applications for automated systems are available on request**

*Materials provided*
• Working solutions as described above
*Additional materials required*
• Calibrators and controls as indicated below
• 0.9% NaCl

**Manual procedure:**

| | |
|---|---|
| Wavelength: | Hg 546 nm (490 - 550) |
| Temperature: | +25 / +30 / +37°C |
| Cuvette: | 1 cm light path |
| Zero adjustment: | against reagent blank<br>one reagent blank per series only |

| | Blank | Calibrator | Sample |
|---|---|---|---|
| Calibrator/R4 | --- | 20 µl | --- |
| Sample | --- | --- | 20 µl |
| R1 | 1000 µl | 1000 µl | 1000 µl |

Mix, incubate for 5 min. at +37°C or 10 min at +20°C or +25°C.
Read absorbance of the sample against reagent blank within 30 min.

**Calculation:**

By calibrator:

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ calibrator}} \times \text{calibrator conc.} = \text{Uric acid conc.}$$

Unit conversion. mg/dl x 59.5 = µmol/l
mg/dl x 0.059 = mmol/l

### Measuring /reportable range:

0.2 – 20.0 mg/dl (11.9 – 1190 µmol/l)

Determine samples with uric acid concentrations > 20 mg/dl via the rerun function. On instruments without rerun function, manually dilute the samples with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. factor 2).

### Expected values:

*Serum/plasma:*

| | |
|---|---|
| Male: | 3.4 – 7.0 mg/dl (202.3 – 416.5 µmol/l) |
| Female: | 2.4 – 5.7 mg/dl ( 142.8 – 339.2 µmol/l) |

*Urine*
(Reference range according to Krieg and Colombo)

| | |
|---|---|
| Morning urine | 37 – 92 mg/dl (2200 – 5475 µmol/l) |
| 24 hour urine | 200 – 1000 mg/24h (1200 – 5900 µmol/l/24h) |
| corresponding to | 13 – 67 mg/dl* (773 – 3986 µmol/l*) |

*Calculated from a urine volume of 1.5 l/24h

*Urine* (Reference range according to Tietz)

| | | |
|---|---|---|
| Average diet: | | 250 – 750 mg/24h |
| Low purine diet: | Male: | < 480 mg/24h |
| | Female: | < 400 mg/24h |
| High purine diet: | | < 1000 mg/24h |

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the uric acid results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

### Analytical sensitivity (lower detection limit):

Detection limit: 0.2 mg/dl (11.9 µmol/l)
The lower detection limit represents the lowest measurable uric acid concentration that can be distinguished from zero.

# Fluitest® UA

**URIC ACID**

analyticon

## Imprecision:

*Serum*

Reproducibility was determined using human samples and controls (n = 20). The following results were obtained:

| | Within run | | |
|---|---|---|---|
| Sample | Mean mg/dl | SD mg/dl | CV % |
| Sample1 | 4.23 | 0.03 | 0.74 |
| Sample2 | 5.96 | 0.04 | 0.65 |
| Sample3 | 11.49 | 0.06 | 0.48 |

Reproducibility was determined using human samples and controls (n = 20). The following results were obtained:

| | Between day | | |
|---|---|---|---|
| Sample | Mean mg/dl | SD mg/dl | CV % |
| Sample1 | 4.70 | 0.04 | 0.92 |
| Sample2 | 6.44 | 0.08 | 1.27 |
| Sample3 | 11.03 | 0.13 | 1.20 |

## Method comparison:

A comparison of the Analyticon Fluitest® UA (y) with a commercial obtainable assay (x) gave with 44 samples the following result:

$y = 0.897\ x - 1.149$; $r = 0.9685$

## Quality Control:

Human Control Serum

| | | |
|---|---|---|
| Contronorm® Plus | 5 x 5 ml | #1205 |
| | 20 x 5 ml | #1220 |
| Contropath® Plus | 5 x 5 ml | #1305 |
| | 20 x 5 ml | #1320 |

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

## Calibration:

S1: 0.9% NaCl

S2: Bio Cal® 20 x 3 ml # 1420

Bio Cal® E 10 x 3 ml # 1430

R4: calibrator provided in kit

## Calibration frequency:

Two-point calibration is recommended

- after lot change
- as required following quality control procedures

## Disposal:

Please note the legal regulations.

## Literature: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