# Isolation of Endophytic Fungi from some Medicinal Plants in Meiktila University Campus and their Starch Hydrolysing Activity

[1]May Thu Khin, [2]Thida Myint, [3]Nilar Cho, [4]Myint Myint Than
[1]Dr, Lecturer, Department of Botany, Meiktila University
[2]Dr, Professor and Head, Department of Botany, Meiktila University
[3]Dr, Lecturer, Department of Botany, Meiktila University
[4]Dr, Lecturer, Department of Botany, Yadanabon University

## *Abstract*

In this research paper, the isolation of endophytic fungi were isolated from four medicinal plants, such as *Andrographic paniculata* L, *Catharanthus roseus* (L.) G.Don. *Azadirachta indica* A. Juss and *Morinda angustifolia* Roxburgh. These plants were collected from Meiktila University Campus. Surface sterilization methods were used for the isolation of endophytes from the leaves. A total of twelve endophytes were isolated and tested for starch hydrolyzing activity. All of them, nine extracts were selected as starch hydrolyzing enzyme procedure.
**Keywords**: Endophytic fungi, Starch hydrolyzing activity, Meiktila University Campus

## Introduction

Plants are generally associated with diverse microorganisms. Endophytic organisms are those that colonize the plant internal tissue showing no external sing of infection or negative effect on their host (Schulz and Boyle 2006).

Endophytic fungi are fungi colonize internally plant tissue without giving detrimental effect to the host plant (Petrini 1992, Azevedo 2004). They act as symbiont, mediate plant resistant against biotic stress i.e. pests and disease and abiotic stress such drough and extreme of temperature (Azevedo 1998).

The microfungi are microorganisms (microbes). The similarities between bacteria and fungi are regarded the techniques needed for their study, their physiology and their ecology are such that mycology can be considered as a branch of micrology, and major contributions to the study of fungi now being made by microbiology (Micheal *et al*. 1994).

Microorganisms are enzymes, which have produced on a large scale. Enzymes are biological catalyst. Enzymes are widely used as a reagent in clinical chemistry and industry. Microbial processes in the industry useful beer and wine production (Sarles *et al*. 1956).

Majority of microorganisms live freely in habitats such as soil and water where they are relatively harmless and often beneficial. A small number of microbes, termed parasites, are harbored and nourished by other living organisms called host. A parasitic cause damage to its host that is known as infectious disease. Most microbial parasites are some type of bacterium, fungus, protozoan, worm, or virus (but not algae). A few microorganisms can exist on either free-living or parasitic levels (Talaro & Talaro 1988).

Microorganisms are particularly good sources of enzymes, which have been produced on a large scale. Enzymes are biological catalysts and have been useful for many years brewing, bread making, cheese-making and tanning. One particular use of enzyme is the manufacture of biological washing powders. Enzymes are widely used as reagents in clinical chemistry, forensic sciences and industry. They were often utilized as a components of intact cells or as extracts containing a mixture of many enzyme (Lowrie and Wells 1998).

The object of this research paper are to study the isolation of endophytic fungi from some medicinal plants and their morphological and microscopial characters and to investigate their potential starch hydrolyzing activity.

## Materials and methods

### Collection of plant sample

Endophytic fungi were isolated from six species of medicinal plants. They were collected from Meiktila University Campus. The collected plants were identified according to the morphological characters shown in literature of Hooker (1885), Backer (1965), Hutchinson (1967) and Hundley (1987). Healthy and mature plants were carefully chosen for sampling. Leaves from each plant were randomly collected for the study. The plant material was brought to the laboratory in sterile bags and processed within a few hours after sampling. Fresh plant materials were used for isolation works to reduce the chance of contamination.

### Isolation of endophytes

In the isolation of endophytic organisms, twelve strains of endophytes were screened from the leaves of

six species of medicinal plants. The methods of isolation of endophytic fungi were referred by the method of (Tomita 1998) and the following procedure.

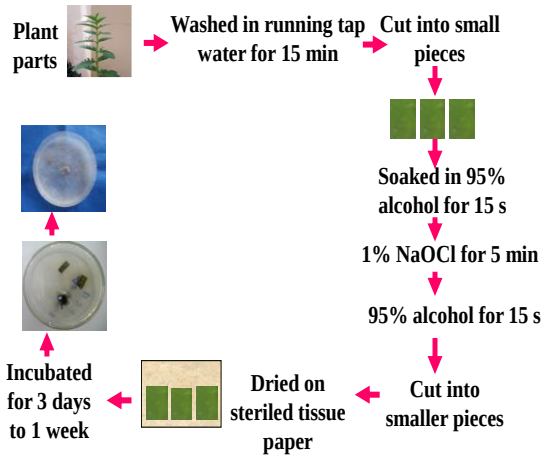


**Figure 1. Isolation procedure of endophytic fungi**

Hyphal tips of the fungus which protruding from the small pieces of leaf on the plate were further purified. PDA (Potato Dextrose Agar) media is used for the purification and the composition is Distilled water 100 ml and PDA 3 g. After autocalving, chloramphenicol was added to the medium. Morphological and microscopial characters were observed by the method of Barnett, 1962 and Ando & Inaba, 2004. For the study of morphology characters PDA media was employed and incuabed for 3 – 7 days at ± 26° ° C temperature.

### Amylase enzyme activity test for starch hydrolyzing

Isolated microorganisms were inoculated into different test tubes containing 10 ml of starch medium and incubated for 3 days. Iodine solution was then poured onto the liquid culture medium. Control tube without microorganisms was also included then the changes in color were studied. Purple color indicated that the microorganisms possesses no starch hydrolyzing enzyme change in color from purple to colorless indicated that the present of microorganisms which contains starch hydrolyzing enzyme.

## Results

### Outstanding features of taxonomic description

Family - Acanthaceae (Figure 2. A)
Scientific Name - *Andrographic paniculata* Nees.PlantaeAsiaticae Rariories 3 1832.
Myanmar Name - Sega gyi
English Name - King of Bitters

Annual erect herbs; stems and branches quandrangular, usually tumid at the nodes. Leafblades lanceolate, cuneate at the base, entire along the margin, acute at the apex. Inflorescences terminal or axillary, racemes or panicles. Flowers bisexual, zygomorphic, white ro pale purple. Calyx campanulate, 5-lobed; segment triangular, glandular-hairy. Corolla bilabiate with 5 lobes; tube swollen at the base; upper lip decurved at anthesis with 2 lobes; lower lip 3-lobed, pale purple with dark purple spots within. Stamens 2, free, far-exserted; filaments filiform, anthers dithecous, longitudinal dehiscence, dark purple. Ovary ellipsoid, bilocular with many ovules in each locule on the axile placentae. Capsules oblongoid.

Family - Apocynaceae (Figure 2. B)
Scientific Name - *Catharanthus roseus* (L.) G.Don. Gen. Syst. 4:95.1837.
Myanmar Name - Thin baw ma hnyo pan
English Name - Madagscar

Perennial herbs with milky latex, slightly fetid. Leaves simple, opposite and decussate, exstipulate, short petiolate; blades elliptic, mucronate at the apex, entire along the margin, cuneate at the base, glabrous on both surfaces, reticulate venation. Inflorescences terminal and axillary cyme. Flowers ebracteate, pedicellate, ebracteolate, bisexual, actinomorphic, pentamerous, hypogyonous, white. Sepals 5, lanceolate, persistent. Corolla 5-lobed, salver form, corolla tube distinctly swollen at the top. Stamens 5, epipetalous, inserted; filaments short; anther dithecous, longitudinal dehiscence. Ovary superior, bicarpellary, bilocular with one ovule in each locule on axile placentae; style long, filiform; stigma subcapitate, green. Fruits linear, follicles.

Family - Meliaceae (Figure 2. C)
Scientific Name - *Azadirachata indica* A. Juss (Neem.) Mus. Hist. Nat Paris. 19:221. 1930.
Myanmar Name - Tama
English Name - Beed tree

Perennial gumiferous, trees. Leaves imparipinnately compound, alternate, exstipulate. Leaflets 9 – 16, elliptic-lanceolate, acute and oblique at the base, serrate along the margin, acuminate at the apex. Inflorescences axillary panicles in the topmost leaf axils. Flowers bisexual, actinomorphic, white. Calyx campanulate with 5 short lobes. Petals 5, oblong or suboblanceolate. Stamens 10, monadephaous, anthers sessile. Ovary globoid or ovoid, 2 to 5 locular, one or two ovules in each locule on the axile placenta. Drupes oblongoid, milky-juicy while young, yellow when ripe.

Family - Rubiaceae (Figure 2.D)
Scientific Name - *Morinda angustifolia* Roxburgh, Pl. Coromandel 3:32. 1815.
Myanmar Name - Yeyo
English Name - Morinda

Perennial large shrubs or small trees, evergreen, sparsely branched. Stem and branches quadrangular, solid, often narrowly fistular. Leaves simple, opposite and decussate, stipulate; stipules interpetiolar, broadly obovate, pale-green, coriaceous; herbaceous; petioles

slightly flattened above, pubescent; blades elliptic to broadly elliptic, acute to cuneate at the bade, entire along the margin, acute to cuneate at the base, entire along the margin, acute at the apex, coriaceous-herbaceous, glabrous on both surfaces. Inflorescences axillary or terminal globose head, many-flowered. Flowers white, capitular, bisexual, actinomprohic, pentamerous, epigynous. Calyx cup-shaped, 5-lobed, without distinct lobes. Corolla tubular, 5-lobed, corolla-tube cylindrical, narrowly beneath and widely above, villous in the throat within; lobes oblong or elliptic-oblong, acute at the apex. Stamens 5, free, adnate to the corolla-tube, inserted; filaments filiform, anthers dithecous, linear, dorsifixed, introrse, creamy or pale yellow, dehiscing longitudinally. Ovary inferior, immersed in the calyx tube, glabrous or obovoid, bilocular, one ovule in each locule on the axile placentae; style slender; pubescent; stigma bifid. Fruits sorosis, ovoid or oblong.

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**Figure 2.** (A) Habit of *Adrographic paniculata* Nees.
(B) Habit of *Caranthus roseus* (L.) G. Don.
(C) Habit of *Azadirachta indica* A. Juss.
(D)Habit of *Morinda anguistifolia* Roxburgh.

**Isolation of endophytic fungi**

Twelve strains of endophytic fungi were isolated from leaves of *Andrographic paniculata* Nees. (Acathaceae), *Catharanthus roseus* (L.) G.Don (Apocynaceae), *Azadirachta indica* A.Juss. (Meliaceae) and *Morinda angustifolia* Roxburgh (Rubiaceae). They were designated as MT - 01 to Mt - 12 respectively. The identification of endophytic fungi was carried out on conventional morphological characteristics and based on the literature of (Ando and Inaba 2004, Barnett 1962). These endophytic fungi were grouped as the fungi imperfecti

**Table 1. Isolation of microorganisms from plant samples**

| Strain No. | Source Plant | Strain No. | Source Plant |
|---|---|---|---|
| MT - 01 | *Andrographic paniculata* | MT - 07 | *Catharanthus roseus* |
| MT - 02 | *Andrographic paniculata* | MT - 08 | *Catharanthus roseus* |
| MT - 03 | *Andrographic paniculata* | MT - 09 | *Catharanthus roseus* |
| MT - 04 | *Andrographic paniculata* | MT - 10 | *Azadirachta indica* |
| MT - 05 | *Andrographic paniculata* | MT - 11 | *Morinda angustifolia* |
| MT - 06 | *Andrographic paniculata* | MT - 12 | *Morinda angustifolia* |

MT – 01 was observed that after 5 days cultivation mycelium surface in white color, colony 4.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long, conidia elliptical, phragmo conidium, production drope.

MT – 02 was observed that after 5 days cultivation mycelium surface in white color, colony 4.0 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long, conidia oblong-elliptical, phragmo conidium, production drope.

MT – 03 was observed that after 5 days cultivation mycelium surface in white color, colony 4.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long, conidia oblong-elliptical, didymo conidium, production drope.

MT – 04 was observed that after 5 days cultivation mycelium surface in white color, colony 5.0 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long, conidia phragmo conidium, production drope.

MT – 05 was observed that after 5 days cultivation mycelium surface in white color, colony 5.0 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores short, conidia globose, production chain..

MT – 06 was observed that after 5 days cultivation mycelium surface in white color, colony 4.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long or short, conidia globose, production drope.

MT – 07 was observed that after 5 days cultivation mycelium surface in white to brown color, colony 3.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores short, conidia phragmo conidium, production chain.

MT – 08 was observed that after 5 days cultivation mycelium surface in white to brown color, colony 3.7 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long or short, conidia globose, production drope.

MT – 09 was observed that after 5 days cultivation mycelium surface in white color, colony 4.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long or short, conidia amerospore, production drope.

MT – 10 was observed that after 5 days cultivation mycelium surface in white color, colony 2.5 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long, conidia elliptical, phragmo conidium, production single.

MT – 11 was observed that after 5 days cultivation mycelium surface in white color, colony 4.5 cm in diameter ±26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores long or short, conidia globose, production drope.

MT – 12 was observed that after 5 days cultivation mycelium surface in white to red brown color, colony 3.3 cm in diameter ± 26° C temperature on PDA medium. Microscopic observation that hyphae septate, conidiophores short, conidia globose, production chain.

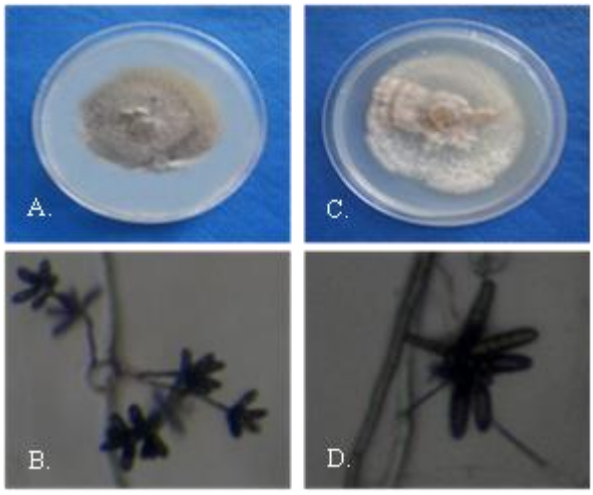


**Figure 3**. A. Morphology of MT-01 isolated from *Andrographic paniculata* Nee.
B. Photomicrograph of MT-01 isolated from *Andrographic paniculata* Nee.
C, Morphology of MT-02 isolated from *Andrographic paniculata* Nee.
D. Pnotomicrograph of MT-02 isolated from *Andrographic paniculata* Nee.

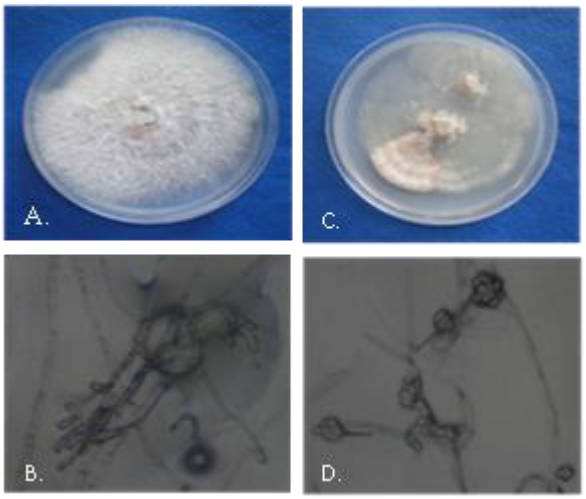


**Figure 4**. A. Morphology of MT-03 isolated from *Andrographic paniculata* Nee.
B. Photomicrograph of MT-03 isolated from *Andrographic paniculata* Nee.
C, Morphology of MT-04 isolated from *Andrographic paniculata* Nee.
D. Pnotomicrograph of MT-04 isolated from *Andrographic paniculata* Nee.

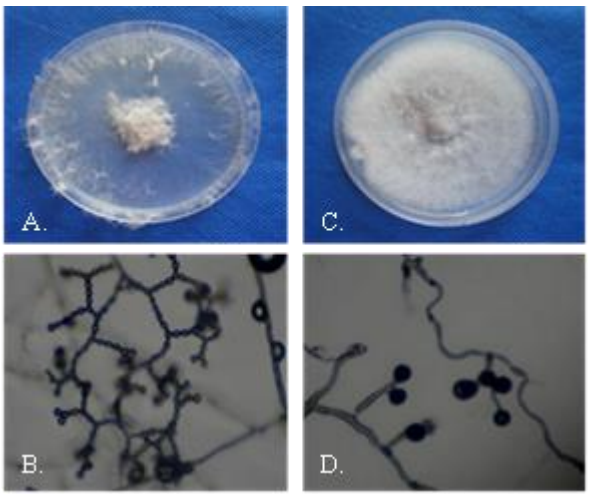


**Figure 5.** A. Morphology of MT-05 isolated from *Andrographic paniculata* Nee.
B. Photomicrograph of MT-05 isolated from *Andrographic paniculata* Nee.
C. Morphology of MT-06 isolated from *Andrographic paniculata* Nee.
D. Photomicrograph of MT-06 isolated From *Andrographic paniculata* Nee.

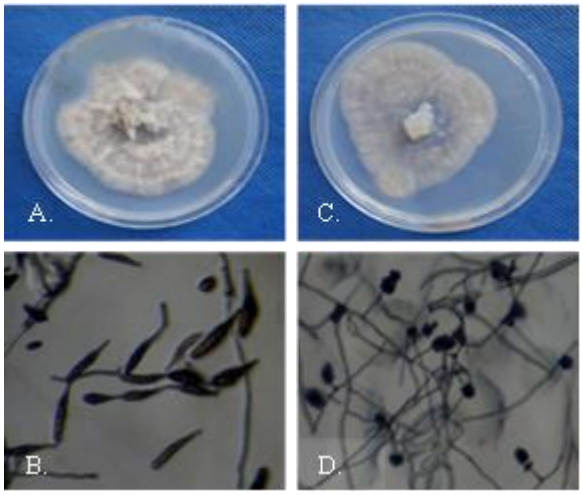


**Figure 6.** A. Morphology of MT-07 isolated from *Catharanthus roseus* (L.) G. Don.
B. Photomicrograph of MT-07 isolated from *Catharanthus roseus*(L.) G. Don.
C. Morphology of MT-08 isolated from *Catharanthus roseus*(L.) G. Don.
D. Photomicrograph of MT-08 isolated from *Catharanthus roseus* (L.) G. Don.

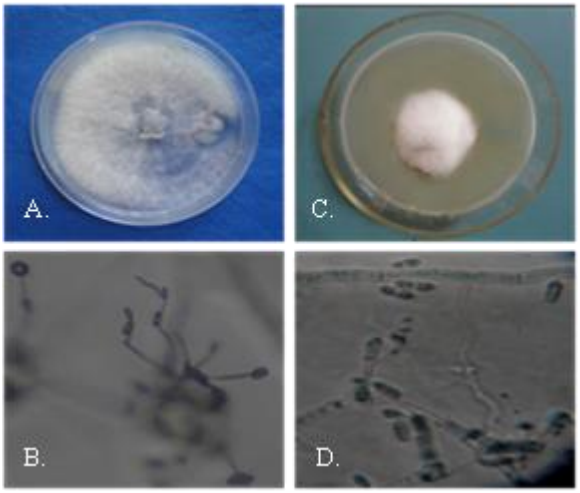


**Figure 7.** A. Morphology of MT-09 isolated from *Catharanthus roseus* (L.) G. Don.
B. Photomicrograph of MT-09 isolated from *Catharanthus roseus*(L.) G. Don.
C. Morphology of MT-10 isolated from *Azadirachta indica* A. Juss.
D. Photomicrograph of MT-10 isolated from *Azadirachta indica* A. Juss.

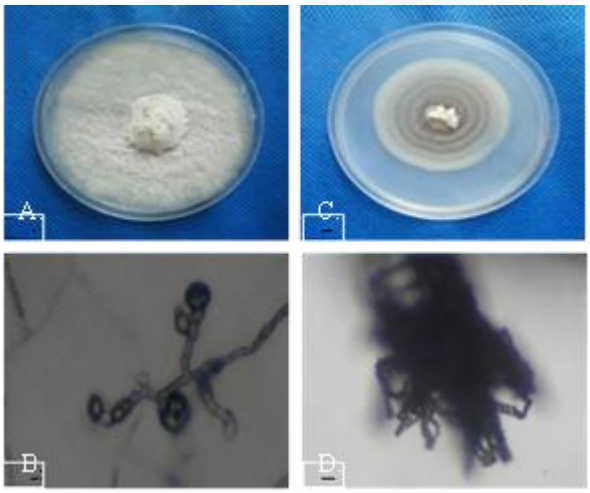


**Figure 8**. A. Morphology of MT-11 isolated from *Morinda angustifolia* Roxburgh.
B. Photomicrograph of MT-11 isolated from *Morinda angustifolia* Roxburgh.
C. Morphology of MT-12 isolated from *Morinda angustifolia* Roxburgh.
D. Photomicrograph of MT-12 isolated from *Morinda angustifolia* Roxburgh.

**Endophytic activity test**

The cultivation of fungi was carried out for 3 days and then checked the enzyme activity with iodine solution by dropping observed that the control tube was changed to purple or blue in colored while strain MT – 01, MT – 02, MT – 03, MT – 04, MT – 05, MT – 06, MT – 07, MT – 08 and MT – 09 were not changed to purple or blue in colored. The strain MT – 10, MT – 11 and MT - 12 were changed purple or blue colored.

Changed color

**Figure 9. Enzyme Activity Test**

## Discussion and conclusion

In this research paper, twelve strains of endophytic fungi were isolated from some medicinal in Meiktila University Campus. These plants were *Andrographic*

*paniculata* (Family Acanthaceae) *Catharanthus roseus* (Family Apocynaceae), *Azadirachta indica* (Family Meliaceae) and *Morinda angustifolia*(Family Rubiaceae). They were designated as MT-01 to MT-12 respectively. In the study of morphological characters, all strains were grown rapidly on PDA media at room temperature about ± 26° C temperature for 3 days to 1 week.

The smallest and largest colonies were 2.5 cm (MT-10) and 5.0 cm (MT-05 and MT-06). In the study of microscopic observation was showed that the hyphae of all strains were septate. The total twelve conidiophores were observed that four short (MT-04, MT-7, MT-10 and MT-12), four long (MT-01, MT-02, MT-03 and MT-04) and four long or short (MT-06, MT-08, MT-09 and MT-11).

The conidia of strains MT-01, MT-02, MT-03 and MT-07 were phargmoconidium, MT-05, MT-06, MT-11 and MT-12 were globose, MT-03 and MT-09 were didymoconidium in shaped. Eight strains (MT-01, MT-02, MT-03, MT-04, MT-06, MT-08, MT-09 and MT-11) of twelve were drope in conidia production. Single conidia production was MT-11 and MT-08 and MT-12 were chain in conidia production.

In enzyme activity test, control tube was changed to purple or blue colored because starch was reacted with iodine. Twelve kinds of endophytic fungi were isolated and tested starch hydrolyzing activity. Among them the strains MT-01, MT-02, MT-03, MT-04, MT-05, MT-06 (*Andrographic paniculata*), MT-07, MT-08 and MT-09 (*Cathranthus roseus*) were starch hydrolyzing enzyme producers. Thus, they were not changed to purple or blue colored. Strains MT-10 (*Azadirachta indica*), MT-11 and MT-12 (*Morinda angustifolia*) were not possessed starch hydrolyzing enzyme. That strains were changed to purple or blue colored. Therefore, nine strains could be used for starch hydrolyzing and three strains could not be used for starch hydrolyzing processes.

So, this research was expected for helping in the study of morphology, isolation of endophytic fingi and their starch hydrolyzing activity from some medicinal plants in further investigation.

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