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Comparative Study on Efficacy of Aloe Vera Leaf and Gel Extracts against *Tinea Corporis* and *Malasezia Furfur*

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Abstract

This study was carried out to determine the phytochemical constituents and comparative antifungal effects of the leaf and gel of Aloe vera (*Aloe barbadensis)* against some dermatophytes. The antifungal efficacy of *Aloe barbadensis* leaf and gel extract was tested against *Tinea corporis* and *Malassezia furfur* using agar well diffusion assays. The phytochemical screening revealed the presence of saponin, alkaloid, tannin, flavonoid and glycoside in both leaf and gel extracts of *Aloe barbadensis*, while steroid was absent in both extracts. Antifungal test was carried out for both leaf and gel extract at 100% concentration. It was observed that the Aloe vera gel extract (GE) showed high activity against *Tinea corporis* and *Malassezia furfur* with zones of inhibition of 10.5±0.2mm and 10.1±0.2mm respectively, while the leaf extract (LE) had zones of 8.7±0.2mm and 6.4±0.1mm for both *Malassezia furfur* and *Tinea corporis* respectively. The positive control (Zinc pyrithione) had slightly higher antifungal effect (10.9±0.5mm) in comparison with that of gel extract (GE) against *Tinea corporis* (10.5±0.2mm) *and Malassezia furfur* (10.1±0.2mm). The MIC of gel extract against both *Tinea corporis* and *Malassezia furfur* was 25%, while the leaf extract had 25% for *Malassezia furfur* and 50% for *Tinea corporis.* The result obtained from the study revealed that both leaf and gel extracts of *Aloe barbadensis* have high antifungal effect against the test organisms and could be potent in the production of alternative new antifungal agents.

Une étude comparative sur l'efficacité des extraits de feuille d'aloevera et de gel contre Tinea corporis et Malasezia furfur

Abstrait

Cette étude a été menée pour déterminer les constituants photochimiques et les effets antifungiques tout en comparer de la feuille et du gel d'aloe vera (Aloe barbaderisis) contre certains dermatophytes. L'efficacité antifungiques de l'extrait de feuilles et de gel d'aloe barbaderisis, tandis que le stéroïde étaient absents dans les deux extraits. Un test antifungique a été réalisé pour l'extrait de feuille et de gel a une concentration de 100%.il a été observe que l'extrait de gel d'aloevera présentait une activité élevée contre Tinea corporis et Malasezia furfur avec des zones d'inhibition de 10,5±0,2mm et 10,1±0,2mm et 6,4±0,1mm pour Malasezia

furfur et Tinea corporis respectivement. Le contrôle positif (zinc pyrithione) avait un effet antifungique légèrement supérieur (10,9±0,5mm) par rapport à celui de l'extrait de gel contre les organismes d'essai et pourraient être puissants dans la production de nouveaux agents alternatifs.

Introduction

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject of very intense pharmacological studies (Kuzmaa et *al*., 2007; Parekh *et al.,* 2007). Higher plants continue to be a rich source of therapeutic agents since they produce hundreds to thousands of diverse chemical compounds as secondary metabolites with different biological activities. The compounds produced by the plants have been reported to be active against plant and human pathogenic microorganisms (Vivek *et al*., 2018).

Yeast like lipophilic basidiomycetous fungus *Malassezia furfur* (*Pytirosporum ovale)* is the causative organism for dandruff (Arora *et al*., 2011). Dandruff is a scalp disorder which is characterized by excessive shedding of skin cells from the scalp. It is a common problem faced by people of all age groups. *Malassezia furfur* converts the sebum lipid into fatty acids and triglycerides, which accelerate hyper-proliferation of keratinoytes (Singla *et al*., 2011).

Dandruff has worldwide occurrence and its frequency depend on different climatic, occupational and socio-economic conditions. It is reported that approximately 30% of dermatophilic infections are due to the lipophilic yeasts. It was observed that the main symptom of dandruff infection was the appearance of patches of discoloured skin with sharp borders and fine scales. The patches were dark reddish-tan in colour. The most common sites were found to be the back, head, underarms, upper arms, chest and neck. Larger lesions were multihued and had relatively sharp irregular margins and smaller lesions were circular or oval (Rai and Sonali, 2009).

Tinea corporis (also known as ringworm) is a superficial fungal infection (*dermatophytosis*) of the arms and legs, especially on glabrous skin; however, it may occur on any part of the body (Bolognia *et al*., 2007). The disease can also be acquired by person-to-person transfer usually via direct skin contact with an infected individual (Likeness, 2011). Animal-to-human transmission is also common (James *et al*., 2006). The fungus can also be spread by touching inanimate objects like personal care products, bed linen, combs, athletic gear, or hair brushes contaminated by an affected person. The *Tinea corporis* fungus infects damaged areas and appears as tiny white threads which multiply into tuffs. The other symptoms like cloudiness of the arms, legs, nails may also be caused by fungus (Mitesh *et al*., 2018).

The Aloe vera plant has been known and used for centuries for its health, beauty, medicinal and skin care properties (Reuter *et al*., 2008). There are over 300 species of aloe, which grow mainly in the dry regions of Africa, Asia, Europe and America. This plant is similar to broad-leaved cactus and is a permanent, meaty, and juicy plant, which reaches a maximum height of two metres. Surprisingly, water constitutes 96% of Aloe vera gel (Sadrnia and Arjomandzadegan, 2014).The scientific name of Aloe vera is *Aloe barbadensis* miller. It belongs to Asphodelaceae (Liliaceae) family, and is a shrubby or arborescent, perennial, xerophytic, succulent, pea-green colour plant (Sharrif and Sandeep, 2011). The plant has triangular, fleshy leaves with serrated edges, yellow tubular flowers and fruits that contain numerous seeds. *Aloe barbadensis* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids (Park and Jo, 2006).

The compounds found in Aloe vera gel contain polysaccharides that are capable of reducing and restoring inflammation. It also has antimicrobial properties. This plant contains hydroxyl anthracene derivatives and aloe resin A, B and C. Also, glucose, mannose, and cellulose sugars, oxidase, amidases, and catalase enzymes, vitamins B1,

B2, B6, C, and E, folic acid, and minerals such as calcium, sodium, magnesium, zinc, copper and chromium form its other nutrients (in low quantities) (Sadrnia and Arjomandzadegan, 2014).

Herbal medications in particular have seen a revival of interest due to a perception that there is a lower incidence of adverse reaction to plant preparation compound to synthetic pharmaceuticals. *Aloe barbadensis* has been shown to have anti-inflammatory activity, immuno-stimulatory activity and cell growth stimulatory activity (Uzma *et al*., 2011). Specific plant compound such as anthraquinones (Gracia-Sosa *et al*., 2006; Dabai *et al*., 2007) and dihydroxyanthraquinones (Wu *et al*., 2006), as well as saponins (Reynolds and Dweck, 1999) have been proposed to have direct antimicrobial activity.

Dermatophytic infections have been one of the major crises prevalent all over the world. They are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly significant in developing countries due to the relative widespread multiple drug resistance as a result of indiscriminate use of commercial antimicrobial drugs. Consequently, drugs of herbal origin could be used to treat fungal infections. Hence, drug development from plant-based compounds could be useful in meeting this demand for newer and more effective antifungal drugs. This study investigated the comparative antifungal effects of leaf and gel of *Aloe barbadensis* against *Tinea corporis* and *Malassezia furfur*.

Materials and Methods

Sample Collection

The sample *Aloe barbadensis* plant was obtained within Gwallameji community in Bauchi State and transported to the microbiology laboratory of Federal Polytechnic Bauchi, Science Laboratory Technology (SLT) Department in a clean polythene bag.

Preparation of Sample

Aloe barbadensis leaves were collected and washed to remove any debris, dried in the laboratory for two weeks and was ground into powder using mortar and pestle. The powder was sieved using mesh sieve of 0.1mm in diameter to obtain the fine powder of the sample. The powder was then preserved in a sterile bottle until required (Soforowa, 2008).

Collection of the Test Organisms

The pure isolate of the test organisms (*Tinea corporis* and *Malassezia furfur*) were collected from Abubakar Tafawa Balewa University Teaching Hospital Bauchi and was transported to the Department of Science Laboratory Technology Laboratory, Federal Polytechnic Bauchi where they were subcultured and confirmed using standard methods.

Preparation of Media

Potato dextrose agar (PDA), Sabaroud dextrose agar (SDA) and broth were prepared according to manufacturer's instruction, used for the growth and maintenance of test microorganisms as well as the susceptibility test.

Extraction Techniques

Crude Aqueous Leaf Extraction (Hot Water)

Fifty grammes of the powdered leaf sample was weighed into a beaker. 350ml of boiled distilled water was poured into the beaker and stirred. It was closed and kept for 24hours at room temperature for complete extraction. The mixture was then filtered using sterile Whatman No. 1 filter paper. The extract was further concentrated in a water bath at 55^{o}C until water was completely evaporated and the pure extract transferred into a reagent bottle and preserved in the refrigerator for further usage (Mamatha *et al*., 2016).

Aloe barbadensis gel Extraction

Mature, healthy and fresh leaves of *Aloe barbadensis* were washed in the running tap water for 5 minutes and rinsed with sterile distilled water, then dissected longitudinally and the colourless parenchymatous tissue (aloe gel) was scraped out using a sterile knife without the fibres. The gel was ground with distilled water using the mortar and pestle. The extracts were filtered using

Whatman No. 1 filter paper and the filtrate was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and stored in refrigerator at 4°C (Mamatha *et al*., 2016).

Phytochemical Evaluation of *Aloe barbadensis* Leaf and Gel Extracts

Test for Steroids

To a volume of 1ml of the extract, five drops of concentrated H_2SO_4 was added, Reddish brown colouration indicates the presence of steroids (Adetunji *et al*., 2013).

Test for Akaloids

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was formed indicating the presence of alkaloids (Bhagyasri *et al*., 2018).

Test for Glycosides

To a volume of 3ml of the extract, 2ml of chloroform was added; H_2SO_4 was carefully added to form a lower layer. A reddish brown colour at interface indicates the presence of glycosides (Adetunji *et al*., 2013).

Test for Saponins

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth was obtained, indicating the presence of saponin (Bhagyasri *et al*., 2018).

Test for Flavonoids

A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta colour was developed within a minute or two, where flavonoids were present (Bhagyasri *et al*., 2018).

Test for Tannins

Two drops of 5% FeCl was added to 1ml of the extract. A dirty green precipitate indicated the presence of tannins (Adetunji *et al*., 2013).

Assaying the Antifungal Activity by Agar Well Diffusion Method

The leaf and gel extract of *Aloe barbadensis* were tested for antifungal activity against *Tinea corporis* and *Malassezia furfur* using the agar well diffusion method as described by Sarika *et al*. (2013).

For plating, potato dextrose agar was prepared, autoclaved and dispensed into sterilized petri plates. After solidification of the agar, 50µL of *Malassezia* furfur and *Tinea corporis* inoculum from broth culture was added in the centre of the petri plate each using a micropipette and spread evenly on the agar using a sterilized glass spreader. Three wells were bored into the agar at three corners of the plate taking care that the wells did not lie in close proximity to the edges of the petri plate or to each other. Each of the three wells represented the sample, standard and control respectively. Using a micropipette, 50 µL of plant extract (filtrate) was added to the sample well. Similarly, 50µL of Distilled water was added to the well representing control. In the well representing the standard, 50µL of 0.01% zinc pyrithione solution was added. The plates were allowed to stand for one hour to enhance diffusion and then transferred to an incubator set at 37°C. After 48 hours of incubation, the susceptibility of the organisms was determined by measuring the diameter of the zone of inhibition around the wells in millimetre (Sarika *et al*., 2013).

Determination of Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of each extract, the agar well diffusion method was used. Each of the aloe vera extracts (leaf and gel extracts) were then used to establish four different concentrations by dilution with distilled water (50%, 25%, 12.5%, and 6.25%) using a starting concentration of 50%. With the help of a sterile cork borer, four equal wells were created. The different concentrations of aloe vera leaf extract (50µL) were transferred into the well using a micropipette. The plates were allowed to

stand for one hour to enhance diffusion and then transferred to an incubator set at 37°C. After 48 hours of incubation, the diameter of the zone of inhibition around the wells in millimetre was measured (Supreet *et al*., 2016).

Results

The phytochemical analysis carried out on the leaf and gel extract is as shown in Table 1. Saponin, tannin, flavonoid, alkaloid, and glycoside were found to be present in both extract with the exception of steroid which was absent in both. Table 2 shows the antifungal activity of leaf and gel extract of aloe vera (*Aloe barbadensis*) at 100% concentration against *Tinea corporis* and *Malassezia furfur*. It was observed that the aloe vera gel extract (GE) showed high activity against *Tinea corporis* and *Malassezia furfur* with zones of inhibition of 10.5±0.2mm and 10.1±0.2mm respectively, while the leaf extract (LE) had zones of 8.7±0.2mm and 6.4±0.1mm for both *Malassezia furfur* and *Tinea corporis* respectively. The positive control (Zinc pyrithione) had slightly higher antifungal effect (10.9±0.5mm) in comparison with that of gel extract (GE) against *Tinea corporis* (10.5±0.2mm) *and Malassezia furfur* (10.1±0.2mm). The negative control (distilled water) inhibited no fungi growth. Table 3 shows minimum inhibitory concentration (MIC) of both leaf and gel extract of *Aloe barbadensis* against *Tinea corporis* and *Malassezia furfur*. The MIC of the extracts against the fungi ranged from 12.5-50%. The gel extract was seen to show zones of 5.0mm, 2.0mm and 0.5mm at 50%, 25% and 12.5% concentration respectively for *Malassezia furfur,* while zones of 2.0mm, and 1.6mm at 50% and 25% concentration respectively against *Tinea corporis*. The aloe vera leaf extract revealed no zones of inhibition at 12.5% and 6.25% concentrations against both Malassezia *furfur* and *Tinea corporis.* Furthermore, the leaf extract showed zones of 3.0mm and 1.5mm inhibition at 50% and 25% concentration respectively against *Malassezia furfur,* while 1.5mm and 0mm inhibition at 50% and 25% concentration respectively against *Tinea corporis.* The minimum inhibitory concentration of gel extract against both *Tinea corporis* and *Malassezia furfur* was 25%, while the leaf extract had 25% for *Malassezia furfur* and 50% for *Tinea corporis.*

Table 1: Phytochemical Results of Leaf and Gel extracts of *Aloe barbadensis*

Phytochemicals	L.E	G.E
Saponin	+	+
Alkaloid	+	+
Steroid	-	-
Tannin	+	+
Flavonoid	+	+
Glycoside	+	+

Key: L.E: Leaf extract, G.E: Gel extract, +: Positive (Present),-: Negative (Absent).

Table 2: Susceptibility Test for Leaf and Gel Extract (at 100% concentration) of *Aloe barbadensis* against *Tinea corporis* and *Malassezia furfur* with zone of Inhibition in millmetre (mm)

Extracts	S.T.D	T. c	M.f
L.E	10.6±0.3	6.4±0.1	8.7±0.2
G.E	10.9±0.5	10.5±0.2	10.1±0.2
C		0	0

Key: L.E: Leaf extract, G.E: Gel extract, S.T.D: Standard (Zinc pyrithione), C: Control (Distilled water), T.c: *Tinea corporis,* M.f: *Malassiezia furfur*

Table 3: Minimum Inhibitory Concentration of Leaf and Gel Extract of *Aloe babadensis* against *Tinea corporis* and *Malassezia furfur*

Extracts	Concentration (%)	Diameter of Inhibition zone (mm)	
		T.c	M.f
L.E	50%	1.5	3.0
	25%	0	1.5
	12.5%	0	0
	6.25%	0	0
G.E	50%	2.0	5.0
	25%	1.6	2.1
	12.5%	0	0.5
	6.25%	0	0

Key: L.E: Leaf extract, G.E: Leaf extract, T.c: *Tinea corporis,* M.f: *Malassezia furfur*

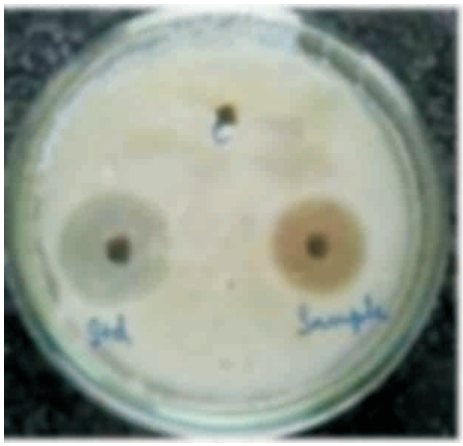

Figure 1

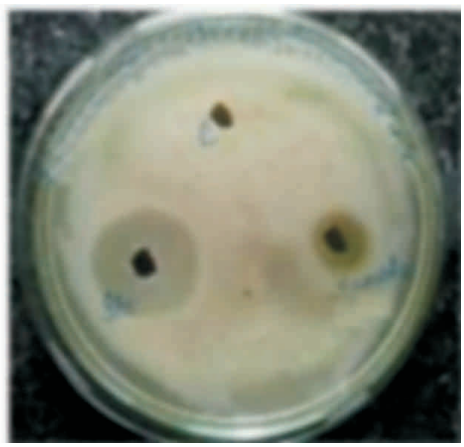

Figure 2

Figure 1 shows the zone of inhibition of aloe vera gel extract against *Tinea corporis*, while figure 2 shows zone of inhibition of aloe vera gel extract against *Malassezia furfur*.

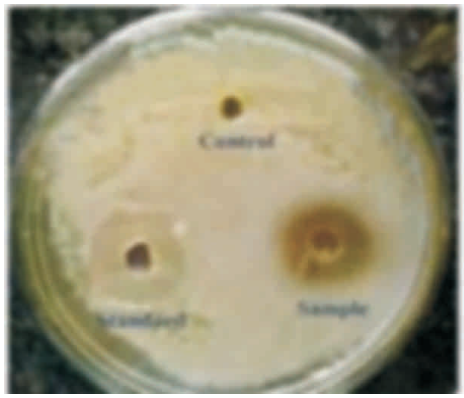

Figure 3

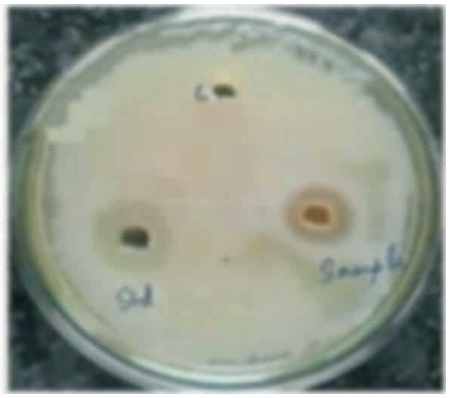

Figure 4

Figure 3 shows the zone of inhibition of aloe vera leaf extract against *Malassezia furfur*, while figure 4 shows zone of inhibition of aloe vera gel extract against *Tinea corporis*.

Key: Each plate has C-Control (Distilled water), 2nd C-Control (Zinc Phythione), Sample-Test organism.

Discussion

The phytochemical screening in Table 1 reveals the presence of tannins, saponins, flavonoids, alkaloid and glycoside in both leaf and gel extracts while steroid was absent in both extracts. These compounds have been reported to have antimicrobial activity and their presence in this plant extract might be the reason for the activity recorded against this test organism. Tannin has been reported to interfere with microbial cell protein synthesis and is important in the treatment of ulcerated or inflamed tissues and also in the treatment of intestinal disorders (Igbinosa *et al*, 2009). Alkaloid has also been reported to be a pain killer and saponin has managing effect against inflammation (Igbinosa *et al*, 2009; Hussain e*t al*; 2009). Flavonoid is also important against inflammation and microorganisms. This is in line with the work of Vivek *et al*. (2018), who reported that herbal medicinal plants possess inherent ingredients (secondary metabolites) that make them effective in treating ailments. Parekh *et al*.,(2007) also reported that plants contain bioactive substances that occur naturally in them. Ibrahim *et al*., (2011) working on phytochemical analysis and antimicrobial evaluation of Aloe vera gel against some human and plant pathogens, also reported that Aloe vera contain these bioactive substances that occur naturally in them.

The antifungal screening of Aloe vera extracts against the test organisms showed zones of inhibition ranging between 6.4mm and10.5mm (Table 2). It was however observed that the Aloe vera gel extract showed slightly higher activity against the fungi with zones of inhibition of 10.5±0.2mm and 10.1±0.2mm for both *Tinea corporis* and *Malassezia furfur* respectively, while leaf extract had zones of 8.7±0.2mm and 6.4±0.1mm for both *Malassezia furfur* and *Tinea corporis* respectively. The study showed that the positive control (Zinc pyrithione) had almost same antifungal effect with that of gel extract against *Tinea corporis and Malassezia furfur.* This is similar to the report of Agarry *et al*. (2005), who worked on Comparative antimicrobial activities of aloe vera gel and leaf and also reported aloe vera leaf and gel to be active against *Tinea corporis* and *Malassezia furfur.* Also, Ibrahim *et al*., (2011) reported aloe vera gel to be active against *Tinea corporis* and *Malassezia furfur.* Shamim *et al*., (2004) noted high zone of inhibition with ethanol extracted from *Aloe baradenisis* against *Candida* species. The study by Suleyman and Sema (2009) similarly reported that *Aloe barbadensis* juice has antimicrobial activity against *Mycobacterium smegmatis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Micrococcus luteus*, *Candida albicans* and *Bacillus sphricus*. Casian *et al*., (2007) stated that hydroalcoholic extracts of fresh leaves of Aloe vera have inhibitory effect against the mycelial growth of *Botrytis gladiolorum*, *Fusarium oxysporum*, *Heterosporium pruneti* and *Penicillium gladioli*. Jasso *et al*., (2005) also evaluated antifungal activity of pulp and liquid fraction of *Aloe barbadensis* on the mycelium development of *Rhizoctonia solani*, *Fusarium oxysporum* and *Collectotrichum coccodes* and found positive results.

However, Sarika *et al*., (2013) working on herbal extracts and their antifungal activity against *Malassezia furfur,* reported that only gel extract of aloe vera was significantly active against *Malassezia furfur,* while the leaf was not.

The minimum inhibitory concentration (MIC) of the gel and leaf extract against *Tinea corporis and Malassezia furfur* ranged from 50% to 12.5%. This is in line with the work of Ibrahim *et al*. (2011) who reported aloe vera gel MIC range of 50% to 6.25% against some human and plant pathogens. In contrast, Mitesh (2018) working on *in-vitro* inhibition of *Tinea Corporis* from various extracts of Aloe vera and *Azadirachta indica*, reported MIC ranges of 75% to 50%.

In this study, aloe vera leaf and gel extracts have shown an effective and good antifungal activity as compared to a commercially available agent. This reveals that the extract could be used against Dandruff causing *Malassezia furfur* and ringworm causing *Tinea coporis*. Use of natural product is not only cost effective but also of negligible side effects (Nair *et al*., 2018).

Nigeria is rich heritage for cultivation and production of herbal medicines due to its diversified climatic conditions. Nigerian traditional literature and ethanopharmacological studies present a number of plants/ formulations with proven efficacy against diseases causing microorganisms (Abubakar *et al*., 2017; Chukwuma *et al*., 2015).

Conclusion

The results of the study showed that gel and the leaf aqueous extracts of aloe vera have effective fungal Inhibition against *Tinea corporis* and *Malassezia furfur.* In addition, Aloe vera gel extract showed more potent antifungal activity as compared to the leaf extract. These results further confirm the therapeutic potency of these plants which are being used as traditional medicine.

Overall, the present study analysed the antifungal effect of Aloe vera leaf and gel extracts against *Tinea corporis* and *Malassezia furfur* and suggests its use for preparation of economic, natural and safe antifungal drugs, which can be used as an alternative for expensive allopathic medicine in treatment of ringworm and dandruff diseases.

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