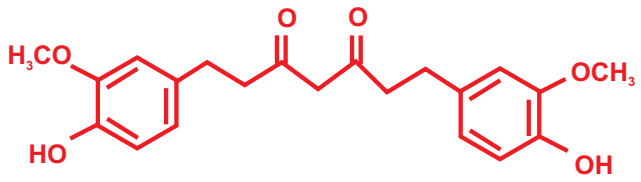
TETRAPURE®.

SKIN. HAIR. NAILS. NATURALLY.

By Muhammed Majeed, Ph.D.*, Pritee Paliwal, Ph.D.** and Anurag Pande, Ph.D.*

TetraPure® is standardized for a minimum of 99% of Tetrahydrocurcumin (1, 7-Bis(4-hydroxy-3-methoxyphenyl)-3,5-heptanedione).

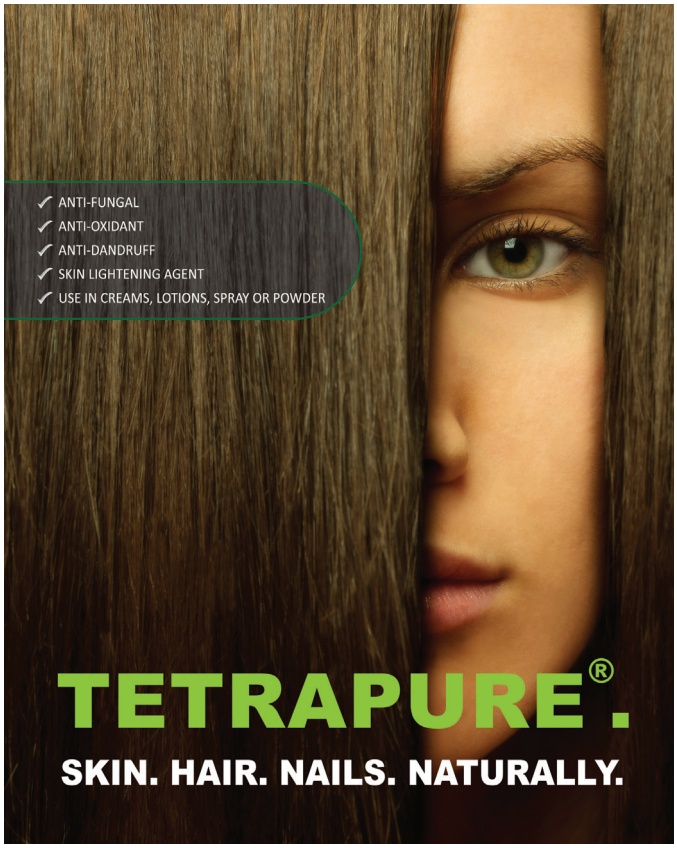


It is known to exhibit similar physiological and pharmacological actions as curcumin, in some cases even better.

Sabinsa's patent (US 8119696) emphasizes the role of TetraPure® ((1,7-BIS(4-HYDROXY-3-METHOXYPHENYL)-3,5-HEPTANEDIONE)) in the management of superficial and cutaneous mycosis. A mycosis (plural - mycoses) is a fungal infection affecting both humans and animals. Approximately 90% of fungal skin infections are caused by 'dermatophytes', which are parasitic fungi affecting the skin, hair and nails. In fact, fungal infection of nails is the most commonly occurring mycoses. Skin is affected by fungus because it feeds on keratin. In hair, fungal invasion often occurs on the hair shaft. It is manifested as itchy, scaly flakes of the scalp (dandruff) or inflammatory abscess that may result in permanent hair loss. People on strong antibiotics are also at risk of fungal infections, as antibiotics kill not only damaging bacteria, but healthy bacteria as well. This alters the balance of microorganisms and results in an overgrowth of fungus.

TetraPure®, is an excellent ingredient for cosmetic formulations with proven efficacy against a wide range of fungi. It can also be a potential natural replacement for anti fungal agents and topical steroids, which often are not free from side effects. TetraPure® can be used in cream, shampoo, ointment, lotion, spray, or powder form for topical application in hair care, skin care and nail care formulations.

Laboratory studies have shown that TetraPure® can be used for the management of superficial and cutaneous mycoses as well as with multifunctional topical benefits such as antioxidant and skin



lightening properties. TetraPure®, has been proven safe for topical use with no irritant or sensitization side effects. It can effectively relive the skin discomforts caused by fungal infections and offer a soothing solution to relieve redness, irritation, scaling, itching, and burning associated with fungal infections.

1. TetraPure®: Treatment of superficial and cutaneous mycosis (fungal infection)

Human mycoses may be broadly classified based on the location of the infection within the body (i) Superficial, cutaneous and subcutaneous; and (ii) Systemic.

* Sabinsa Corporation
** Sami Labs LTD

Superficial mycoses include cosmetic fungal infections of the skin or hair shaft with no involvement of living tissue or cellular (immunological or pathological) responses from the host. Cutaneous mycoses are also superficial fungal infection of the skin, hair or nails. No living tissue is invaded, however a variety of pathological changes occur in the host because of the presence of the infectious agent and its metabolic products. Therapeutic management of superficial and cutaneous fungal diseases has posed a tremendous challenge owing to the fact that fungal cells, like other living organisms, may become resistant to drugs. Antifungal resistance may be defined as a stable, inheritable adjustment by fungal cell to an antifungal agent, resulting in a less than normal sensitivity to that antifungal agent.

Fungal infections are responsible for several disease conditions in human and plant kingdom. Some pathogenic fungi such as *Malassezia furfur* give rise to an unhygienic scaling of the skull. Other systemic infections produce debilitating health conditions such as fever and pain. TetraPure® is novel inhibitor of fungi growth. It can be used in cream, shampoo, ointment, lotion, spray, or powder form to relieve skin discomforts that arise from fungal infections.

• Antifungal Efficacy – Agar Dilution Method (Sabourauds Dextrose Agar, SDA)

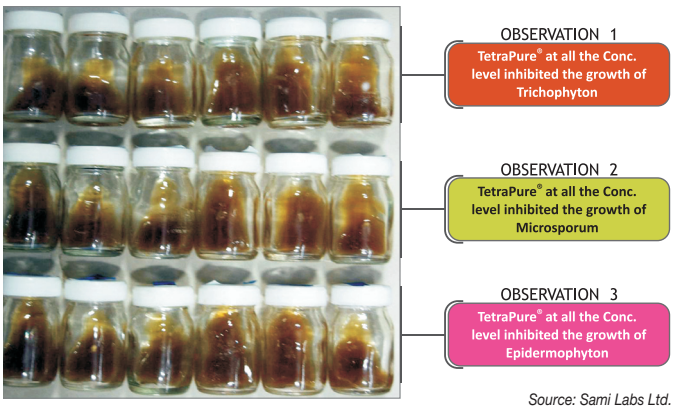
TetraPure® inhibits the growth of dermatophytes *Trichophyton rubrum, Microsporum gypseum* and *Epidermophyton flocossum* at all tested concentrations viz 5%, 2.5%, 1.25%. 0.625%. 0.31% & 0.15% (Table 1).

Test Organism	TetraPure® in DMSO							DMSO	SDA
	Trial	5%	2.5%	1.25%	0.625%	0.31%	0.15%	Control	Control
Trichophyton rubrum	TR1	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	TR2	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	TR3	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
Microsporum gypseum	MG1	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	MG2	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	MG3	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
Epidermophyton flocossum	EF1	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	EF2	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth
	EF3	N/G	N/G	N/G	N/G	N/G	N/G	Growth	Growth

Source: Sami Labs Ltd. (N/G= NO GROWTH)

Table 1: Antifungal Efficacy of TetraPure®

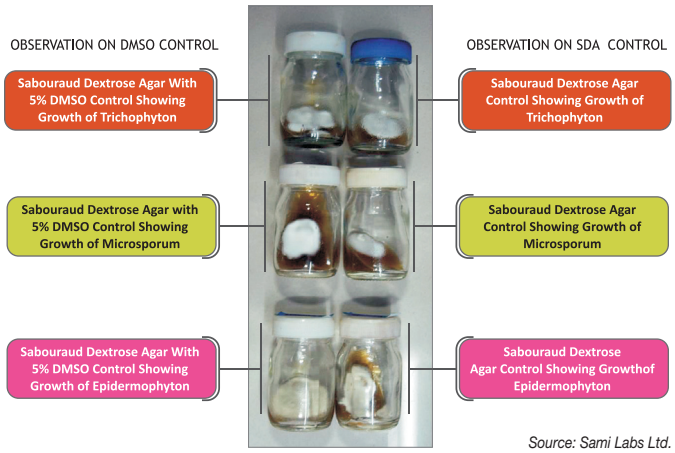
Figure 1a shows the anti-dermatophytic activity of TetraPure®. TetraPure® at concentrations of 0.15%, 0.31%, 0.625%, 1.25%, 2.5% and 5.0%, completely inhibiting the growth of *Trichophyton rubrum, Microsporum gypseum* and *Epidermatophyton floccosum* both at low and high concentrations.



Source: Sami Labs Ltd.

Fig. 1a: Anti Dermatophytic activity of TetraPure®

Fig 1b shows no effect of DMSO used to dissolve the test compound (TetraPure®) on the growth of dermatophytes *Trichophyton rubrum, Microsporum gypseum* and *Epidermophyton floccosum.*



Source: Sami Labs Ltd.

Fig. 1b: Growth Observed in both control Sabouraud Dextrose Agar (SDA) and 5% DMSO

• Activity against Candida Species:

TetraPure® at 0.15%, reduces the colony counts of *Candida albicans* NCIM3471 (Yeast) to less than 100cfu/ml with an overall percentage reduction of 99.99% over a test interval time of 28 days (Table 2).

Test Organism	Test interval (days)	Concentration of organism (cfu/ml)	% Reduction
Candida albicans **NCIM 3471 (yeast)**	0	14.6×10^5	---
	7	72×10^5	50.6
	14	20×10^8	99.8
	21	75×10^2	99.94
	28	<130	99.99

Source: Sami Labs Ltd.

Table 2: Activity Against Candida species

2. TetraPure® – Antioxidant Potential

TetraPure® offers effective topical antioxidant protection. Its antioxidant action is of a comprehensive "bioprotectant" nature, efficiently preventing the formation of free radicals, while quenching pre-formed ones as well. This dual action protects the skin cells from damage by UV radiation and the resultant inflammation and injury with far reaching beneficial effects on overall health and well being. *In vitro* data reveal that TetraPure® efficiently scavenges free radicals and protects the skin cells. The free radical scavenging activity of TetraPure® was found to be superior to that of the Vitamin C, kojic acid and arbutin (Table 3). TetraPure® offers protection to the skin and could be included in as functional antioxidants in topical preparations.

Product	DPPH Inhibition (IC_{50}µg/ml)	ORAC Value (µmol Trolox equivalents/g)
TetraPure®	1.3	10,212
Vitamin C	1.93	3,400
Kojic Acid	500	Nil
Arbutin	500	Nil

Source: Sami Labs Ltd.

Lower the IC_{50} value, greater is the Antioxidant Potential.
Greater the ORAC value, better is the Antioxidant Potential.

Table 3: Comparison of Antioxidant Potential

3. Anti-dandruff activity (Activity against *Malassezia furfur* (MF) – Agar Dilution Method)

TetraPure® inhibited the activity of *Malassezia furfur* at all tested concentration (Table 4).

Test Organism	TetraPure® DMSO solvent with final concentration						
	Trial	5%	2.5%	1.25%	0.625%	0.31%	0.15%
Malassezia furfur	MF1	N/G	N/G	N/G	N/G	N/G	N/G
	MF2	N/G	N/G	N/G	N/G	N/G	N/G
	MF3	N/G	N/G	N/G	N/G	N/G	N/G

Source: Sami Labs Ltd. (N/G= NO GROWTH)

Table 4:Activity Against Malassezia furfur

4. TetraPure® – Skin Lightening Potential

In vitro studies indicate that TetraPure® efficiently inhibits tyrosinase, the rate limiting enzyme in the synthesis of melanin. Its efficacy is superior to that of commonly used natural skin lightening agents such as kojic acid, and of related compounds (Table 5).

Product	Tyrosinase Inhibition (IC_{50}µg/ml)	Melanin Inhibition (IC_{50}µg/ml)
TetraPure®	1.8	3.2
Vitamin C	9.33	25
Kojic Acid	7	100
Arbutin	193.6	100

Source: Sami Labs Ltd.

Lower the IC_{50} value, greater is the Skin lightening activity.

Table 5: Comparison of Skin Lightening Activities

5. Safety studies of TetraPure®

Dermal Irritation
- Non-Irritant

Repeated insult patch test
- Did not induce skin sensitization or any reaction

Primary Skin Irritation
- Did not cause any irritation

6. Suggested use level

7. In Anti-fungal/ Anti-dandruff formulation : 0.15%w/w

8. In Skin lightening/anti-oxidant formulation: 0.1 - 0.5%w/w

"TetraPure® effectively relieves skin discomforts due to fungal infections and offers a balmy resolution to soothe redness, irritation, scaling, itching, and blazing compared with fungal infections, and can be used in all ranges of cosmetic products for progressing healthy skin, hair and nails"

References

- *Majeed M, (2012). Treatment of superficial and cutaneous mycoses with a pure form of 1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-heptanedione. US 8119696.*
- *Research Report-Sami Labs Ltd. (2003). Primary skin irritation test, Report # 8954.*
- *Research Report-Sami Labs Ltd. (2003). Repeated insult patch test, Report # AACP/PP/163.*
- *Research Report- Sami Labs Ltd. (2006). Report on Dermal Irritation, Report # IR 161*