TABLE 1 : ANALYSIS OF TERAZOSIN TABLETS

Formulation	Labeled amount (mg)	Amount found Proposed method[A] (mg)	Amount found UV-method[B] (mg)	% Recovery[C]
Tablet sample-1	5	5.01	5.04	100.18
Tablet sample-2	1	0.996	1.03	100.12

A denotes average of five determinations. B represents tablet sample was dissolved in methanol, water hydrochloric acid (3000:9000:9) and filtered before the absorbance was measured at 246nm. C denotes recovery of 1mg pure drug added to tablet sample preparations.

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REFERENCES

1. Reynolds, J.E.F., Eds., In; Martindale The Extra Pharmacopoeia, 29 th Edn., Council of Royal Pharmaceutical Society of Great Britian, London, 1989,502.
2. Budavari,S.,Eds., In; The Merck Index, 12th Edn., Merck and Co. Inc., Whitehouse Station, NJ, 1996, 1563.
3. Baner, J.B. and Krogh, S.K., **J.Chromatogr.**,1993, 648, 175.
4. Sharma, R.K., **J. Chromatogr.**, 1984,36,206.
5. Patterson, S.K., **J. Chromatogr.**, 1986, 15, 260.

Antifungal Activity of *Calendula officinalis*

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The *in vitro* antifungal activity of *Calendula officinalis* flower extracts have been investigated against *Aspergillus niger, Rhizopus japonicum, Candida albicans, Candida tropicallis* and *Rhodotorula glutinis.* The extracts of *Calendula officinalis* showed high degree of activity against all test fungi. The inhibitory effects of extracts are very close and identical in magnitude and are comparable with that of standard abtibiotics used.

Calendula officinalis Linn. (Compositae) which is also known as marigold, is a herb employed in traditional medicine in many parts of India. The medicinal properties of this plant has been mentioned in Ayurvedic and Unani System of Medicine indicating that the leaves and flower extracts are antipyretic, antiinflammatory, antiepileptic and antimicrobial. It is used internally for fevers to promote perspiration and to prevent suppuration[1]. It is also reported to posses wound healing activity[2]. Infusion of *C. officinalis* is also popularly used in anaemia and in ointment for sores, cuts, bruises and for the treatment of cracks in hands[3]. The present study was undertaken to evaluate antifungal activity of *C. officinalis* extracts.

The *C. officinalis* flower were procured from the

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TABLE 1 : ANTIFUNGAL ACTIVITY OF *CALENDULA OFFICINALIS* EXTRACTS

Organisms	Diameter of growth inhibition zone (mm)					
	Extracts				*Standards*	
	Petroleum ether	Chloroform	Acetone	Ethanol 95%	Amphotericin B	Nystatin
	a	a	a	b	a	a
A. niger	14	18	16	12	20	—
R. japonicum	12	10	10	10	16	—
C. albicans	18	12	14	11	16	20
C. tropicallis	13	12	14	14	16	18
R. glutinis	20	18	12	14	28	25

All values are an average of three determinations. The concetrations of extracts employed were a 1000 µg/ml., b. 20,000 µg/ml. Diameter of cups was 6 mm. Tween 20 alone showed no activity against any of the organisms.

Nagpur Improvement Trust Garden, Nagpur, during the months of July-August. The material was botanically identified and confirmed by the Department of Botany, Nagpur University, Nagpur. The flowers were dried under shade and powdered. The powdered flowers were exhausitvely extracted with petroleum ether (60-80°), chloroform, acetone and ethanol (95%) using a Soxhelt extractor. The extracts were concentrated to dryness *in vacuo*.

The ethanol (95%) extract was dissolved in sterile water while other organic solvent extracts were made soluble in sterile water by using sterile Tween 20 (0.5 ml), which was previously tested for antifungal activity against all test fungi and found negative, followed by dilution to get test solutions of desired concentrations. Solutions of desired concentrations of amphotericin B and nystatin were prepared in dimethyl formamide and used as standard.

The antifungal activity of various organic solvent extracts was assayed by cup-plate method[4]. Amphotercin B and nystatin were used as standard and Sabouraud dextrose agar was employed as medium. The *in vitro* screening of antifungal activity was carried out against 72 h cultures of *A. niger, R. japonicum, C. albicans, C. tropicallis* and *R. glutinis*. The plates were inoculated with 72 h growth of respective fungi in Soyabean casein digest medium. A previously liquified medium was inoculated with 0.2 ml uniformly turbid growth suspension of the test fungi at a temperature of 37-40° and immidiately poured 20 ml of the inoculated medium into the plates having internal diameter of 8.5 cm. The plates were kept on levelled surface to get uniform thickness. After complete solidification of medium, the cups were made aseptically with cork borer having 6 mm diameter and 0.2 ml of test solutions of each extract as well as the standard was poured in it using dropping pipette under aseptic conditions. The plates were kept at 25 - 27° and the zones of inhibition measured after incubation for 72 h. Each experiment was carried out in triplicate and the mean diameter of inhibition zone was recorded.

Results of screening of antifungal activity of *C. officinalis* extracts are summarised in Table 1. It is evident from the results that, various organic solvent extracts showed high antifungal activity against all the test fungi. All four extracts showed significant growth inhibition of *A. niger, R. japonicum, C. albicans, C. tropicallis* and *R. glutinis*. The degree of growth inhibition ranged from 10 mm to 20 mm against test fungi and was comparable with amphotericin B and nystatin, the standard antibiotics employed. The petroleum ether (60-80°), chloroform and acetone extract of *C. officianalis* flowers was found to have wider antifungal activity. From these results, it can be concluded that *C. officinalis* extracts can be regarded as broad spectrum antifunal agents.

The detailed chemical nature of the active principle(s), responsible for antifungal activity is not known. However, the preliminary phytochemical screening has shown the presence of cardiac glycosides, sterols and flavonoids.

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REFERENCES

1. Kadkarni, A.K., In; Indian Materia Medica, 3rd Edn., Vol. I, Popular Book Depot. Bombay, 1954, 234.
2. Rao, S.G., Udupa, A.L., Rao, G., Rao, P.G.M. and Kulkarni, D.R., **Fitoterapia,** 1991, 62, 508.
3. Francesco, B. and Francesco, C., In; Health Plants of the World Atlas of Medicinal Plants, 1st Edn., News Week Books, New York, 1977, 158.
4. Indian Pharmacopoeia, 3rd Edn., Vol. II, The Controller of Publication, Delhi, 1985, A-88.

5-Amino Salicylic Acid Inhibits Nitrite-Induced Methemoglobin Formation

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5 Amino salicylic acid (5-ASA) and several structurally-related analogs were tested for inhibitory role on nitrite-induced oxidation of hemoglobin in human blood-hemolysates. Results indicate a strong inhibitory role for 5-ASA in a concentration range of 0.2 to 0.8 mM. Although the inhibitory activity of 5-ASA was higher than other structural alalogs tested, the activity of curcumin was much higher at equimolar levels. PABA also showed some activity at similar concentrations although it was lower than that of 5-ASA. Benzoic acid showed marginal activity at lower concentrations. On the other hand, acetyl salicylic acid and salicylic acid increased the methemoglobin levels in hemolysate.The pro-oxidant activity was higher at higher concentrations for these compounds. Higher level of activity of 5-ASA is consistent with previous findings. As acetyl salicylic acid and 5-ASA are currently being prescribed, it is worth investigating their *in vivo* activity in order to establish novel and hitherto unexplored clinical applications.

Hemoglobin is subjected to severe oxidant stress. When hemoglobin binds to molecular oxygen, there is an accompanied risk of superoxide production along with the oxidation of hemoglobin to methemoglobin[1]. There are several inherent antioxidant defense meachanisms which prevent methemoglobin formation. Superoxide dismutase, catalase, uric acid, ascrobic acid and glutathione peroxidase constitute a few of these endogenous antioxidants[2]. In spite of this, oxidation of hemoglobin to methemoglobin occurs in response to a variety of chemical stimuli which include drugs like primaquine and dapsone and environmental pollutants such as nitrogen dioxide. The endogenous antioxidant defense system present in our body maintains the level of methemoglobin within one percent. Many antioxidants such as ascorbic acid, uric acid, 3-ribosyluric acid and glutathione have been found to protect hemoglobin from nitrite-induced oxidation[3]. Nitrite oxidizes hemoglobin in two stages, viz. a slow stage followed by a rapid autocatalytic stage involving superoxide anion, hydrogen peroxide and nitrogen dioxide. Curcumin, an established free radical scavenger, protects hemoglobin against nitrite-induced oxidation[4,5].

There is a great deal of evidence to show that the antioxidant activity of 5 amino salicylic acid (5-ASA) is

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