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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS FROM *TRIDAX PROCUMBENS* L. AGAINST POTENTIALLY PATHOGENIC FUNGAL SPECIES

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ABSTRACT

Background: The *Tridax procumbens* plant is well known for its traditional medicinal values. The present study deals with evaluation of antifungal activity of the extracts obtained from *Tridax procumbens L.*

Method: Different extracts of the parts like leaf, stem, flower and roots were prepared in five solvent systems of polar and non-polar origin. The antifungal activity was assayed using agar well diffusion method.

Results: All extracts of different parts responded variedly. The methanolic root extract of *Tridax procumbens* was found to be most potent for the *Candida species.* The most susceptible fungi were found to be *Candida albicans* (MTCC 3017), *Candida albicans* (MTCC 227), *Candida glabrata* (MTCC 3019) and *Saccharomyces cerevisiae* (MTCC 170). The highest antifungal activity of methanol root extract with 16 mm inhibition zone was measured against the *Candida glabrata* (MTCC 3019).

Conclusion: The present study concluded that, the plant had significant antifungal potential for the tested *Candida* species and other assayed fungi. The study validates the use of this plant as antifungal agent.

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INTRODUCTION

The fungal infection remains major killer and also a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents (McNeil *et al.*, 2001)**.** The strains of *C. albicans* with multiple antibiotic resistance is increasing throughout world, it is of great importance to develop new effective therapeutics for those resistant strains. Therefore, researchers are increasingly turning their attention to traditionally used medicinal plant, looking for new leads to develop better drugs against microbial infections (Srinivasan *et al.*, 2001)**.** The spectrum of antifungal activity of any particular plant for the common pathogenic fungi lies in its bioactive constituents. Therefore phytochemical screening is guiding key for determining the antifungal activity. The most active phytochemicals present in the plant are alkaloids, tannins, flavonoids, and phenolic compounds (Ali *et al.*, 2001; Hill, 1952)**.** These phytochemicals often termed as secondary metabolites and includes terpenes (such as plant volatiles, cardiac glycosides, carotenoids and sterols), phenolics (such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins and lignin) and nitrogen containing compounds such as alkaloids and glucosinolates (Tania *et al.,* 2012)**.** *Tridax procumbens* (Family: *Asteraceae*) is a medicinal plant, found perennially in various tropical and subtropical regions as well as mildly temperate regions worldwide (Mundada and Shivhare, 2010). The plant is used for diarrhea, malaria, cough and asthma, boils, epilepsy, liquid purging, wounds, toothache and stomachache. Leaves are applied on cuts and wounds and its paste used for the treatment of dysentery. Blend of roots with castor oil applied on paralytic part (Singh *et al*., 2003). The extracts of *T. procumbens* have been reported to have hair growth promoting activity (Saraf *et al.,* 1991)**.**

The plant *Tridax procumbens* have been reported to have various pharmacological effects, antimicrobial activity against both Gram-positive and Gram negative bacteria **(**Sharma and Kumar, 2009; Manjamalai *et al.,* 2010; Jindal and Kumar, 2013; Tejaswini *et al*., 2011). Therefore the present study was aimed for management of the fungal infections and their respective causative agent by the use of active extracts from the *Tridax procumbens* plant.

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MATERIALS AND METHODS

Plant Materials

The plant *Tridax procumbens* L. was collected from different localities of Wardha district of Maharashtra state. Wardha district is located between latitude 20°-21° North and longitude 78°-79° East of Maharashtra state, India. Collection was done during January 2012 to April 2012, from the areas like gardens and farms. The collected plant material was transported soon to the laboratory and washed thoroughly with distilled water. The plant was authenticated in the Department of Botany, Adarsha Mahavidhyalaya, Dhamangaon (Rly). The cleaned whole plant was sorted into different parts of root, stem, flowers and leaf. The parts were allowed for the complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtight container.

Extract Preparation

Dried powdered plant parts (root, leaf, flower and stem) were extracted successfully with the organic solvents with increasing of polarity by using Soxhlet extraction assembly. The extraction was carried out for 24 – 48 h. at room temperature with mild shaking. For the extraction procedure pure solvents of polar and non-polar origin (ethanol, methanol, acetone, chloroform, and ethyl acetate) were used. The powdered finely ground and dried plant material (25 g) of each part was extracted sequentially using Soxhlet extractor with 250 ml of pure organic solvent separately in order to extract non-polar and polar compounds. The obtained crude extracts were then filtered through Whatman No.1 filter paper and bottled in separate containers and concentrated at 40°C using a drier. The concentrated extracts were subsequently dried aseptically at room temperature. Extract was stored in sterile screw cap bottles under refrigeration condition at 4^0 C prior to use for subsequent assays.

Antifungal Assay

For testing antifungal activity the standard test cultures of *Rhizopus oryzae* (MTCC 554), *Aspergillus niger* (MTCC 281), *Aspergillus flavus* (MTCC 277), *Candida albicans* (MTCC 227), *Penicillium chrysogenum* (MTCC 160), *Candida albicans* (MTCC 3017), *Candida tropicalis* (MTCC 184), *Candida glabrata* (MTCC 3019) and *Saccharomyces cerevisiae* (MTCC 170) were used. The cultures were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. Antifungal activity was determined by agar well diffusion method using Sabouraud's dextrose agar. Test fungus was inoculated by streaking the swab over the entire sterile agar surface to get almost confluent lawn of growth after incubation. A sterile stainless steel borer was used to prepare two cups in the agar media. Stock solution for the five crude extracts was prepared with their respective solvents with a concentration of 100 mg/ml of crude extract. To each plate, one bore was filled with 100 µl pure solvent and marked accordingly. To the other bore, 100 µl of the crude extract solution of concentration 100 mg/ml was added. Petri dishes were then incubated at 25^0C for 24 - 48 h. The zone of inhibition was measured using a zone reader and the results were noted by subtracting the zone produced by pure solvent from the zone produced by the test crude extract.

RESULTS

In the present study antifungal potential of *Tridax procumbens* plant was evaluated against different fungal species. The study was carried as per the standard procedure in the Research Laboratory of Department of Microbiology, Adarsha Science J. B. Arts and Birla Commerce, Mahavidhyalaya, Dhamangaon, Railway, District, Amravati. Duration of the study was January 2012 to November 2012. All the microbiological media were procured from the Hi- Media, Private Laboratory, Mumbai. Twenty extracts of root, stem, leaf and flower of *T. procumbens* prepared in five different solvent systems were screened for antifungal activity against nine different fungal species. Results revealed that all the extracts were found inactive against *Rhizopus oryzae* (MTCC 554), *Aspergillus niger* (MTCC 281) and *Aspergillus flavus* (MTCC 277), except slight inhibitory activity of stem and leaf ethanol extract against *Aspergillus niger* (Table 1). Ethanolic stem and flower extract demonstrated significant inhibitory activity against *Candida albicans* (MTCC 227) and *Candida albicans* (MTCC 3017), while *Candida glabrata* (MTCC 3019) shown inhibitory activity with 10 mm zone against ethanol flower extract. The root extract from ethanol showed major activity with 11 mm zone against the *Candida albicans* (MTCC 3017).

Table 1. Antifungal activity of *Tridax procumbens* L. extracts.

Solvent System	Plant part / fungi	*R.oryzae 554*	*A. niger 281*	*A. flavus 277*	*C. albicans 227*	*P. chrysogenum 160*	*C. albicans 3017*	*C. tropicalis 184*	*C. glabrata 3019*	*S.cerevisiae 170*
		Diameter of Zone of inhibition in mm								
Ethanol	Leaf	-	8	-	8	-	8	8	8	8
	Stem	-	9	-	10	-	10	8	9	10
	Flower	-	-	-	10	-	10	10	9	-
	Root	-	-	-	8	-	11	-	8	-
Methanol	Leaf	-	-	-	10	-	11	-	-	11
	Stem	-	-	-	8	-	10	-	-	-
	Flower	-	-	-	11	-	13	-	-	11
	Root	-	-	-	8	-	13	10	16	12
Acetone	Leaf	-	-	-	8	-	8	-	8	8
	Stem	-	-	-	8	-	8	8	9	8
	Flower	-	-	-	10	8	9	9	10	8
	Root	-	-	-	10	8	10	10	10	8
Ethyl Acetate	Leaf	-	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	-
	Flower	-	-	-	8	8	10	8	8	8
	Root	-	-	-	9	8	10	9	10	8
Chloroform	Leaf	-	-	-	-	-	-	-	-	-
	Stem	-	-	-	-	-	-	-	-	8
	Flower	-	-	-	8	8	8	-	10	8
	Root	-	-	-	9	8	9	10	10	10

Methanolic extracts from different parts showed maximum antifungal potency than the ethanolic extract. For *Candida*

albicans (MTCC 227) methanol leaf extract showed significant inhibitory activity with 10 mm zone, while flower extract showed 11mm zone. For another strain i.e. *Candida albicans* (MTCC 3017) inhibitory zones were recorded from methanolic extraction as 11 mm for leaf and 10 mm for stem, while 13 mm zone was recorded for both flower and root extracts. Methanol root extract showed 10 mm zone against the *Candida tropicalis* (MTCC 184), while the same extract showed greater potency for *Candida glabrata* (MTCC 3019) with 16 mm inhibition zone. For *Saccharomyces cerevisiae* (MTCC 170) significant inhibitory effects (11-12 mm inhibition zone) was noted for leaf, flower and root extracted with methanol, while stem extract failed to show any activity. The acetone extract of root showed significant antifungal activity with 10 mm zone for all four tested *Candida* species, i.e *Candida albicans* (MTCC 227), *Candida albicans* (MTCC 3017), *Candida tropicalis* (MTCC 184) and *Candida glabrata* (MTCC 3019), while acetone flower extracts showed 10 mm zone for only two *Candida species* i.e. *Candida glabrata* (MTCC 3019) and *Candida albicans* (MTCC 227). Acetone stem extracts showed inhibitory activity towards *Candida albicans* (MTCC 227), *Candida albicans* (MTCC 3017), *Candida glabrata* (MTCC 3019), *Candida tropicalis* (MTCC 184) and *Saccharomyces cerevisiae* (MTCC 170) with growth inhibition zones ranging from 8 to 9 mm. The acetone extract of four different parts was ineffective towards *Rhizopus oryzae* (MTCC 554), *Aspergillus niger* (MTCC 281) and *Aspergillus flavus* (MTCC 277).

Table 2. Effectiveness of *Tridax procumbens* extract against tested fungal species

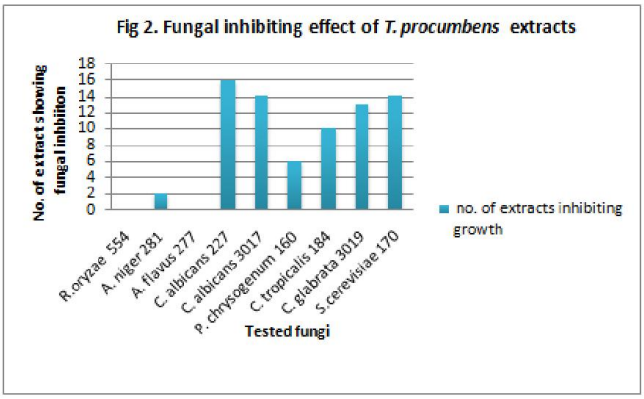
Extract type	Leaves		Stems		Flowers		Roots	
Solvent used	Active	Inactive	Active	Inactive	Active	Inactive	Active	Inactive
Ethanol	6	3	6	3	4	5	3	6
Methanol	3	6	2	7	5	4	5	4
Acetone	4	5	5	4	6	3	6	3
Ethyl Acetate	0	9	0	9	6	3	6	3
Chloroform	0	9	1	8	5	4	6	3

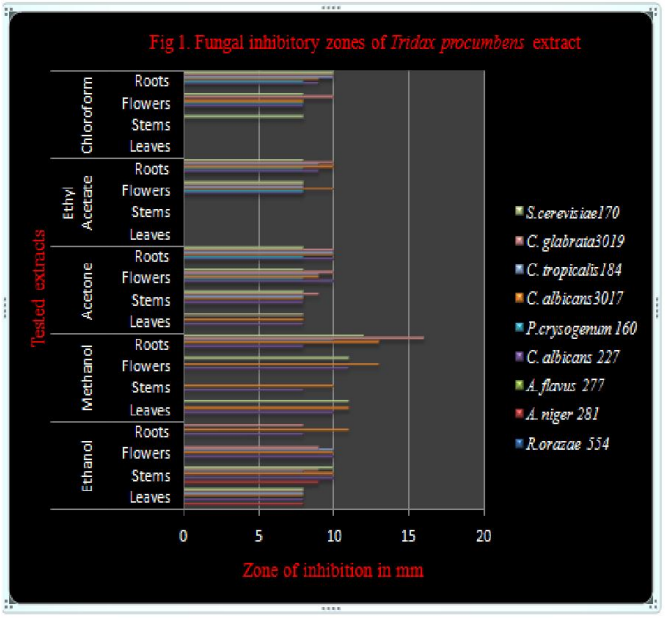
No. of tested fungi = 09

Ethyl acetate and chloroform extract from leaf and stem showed no effect on any fungal species except slight inhibitory effect of stem chloroform extract on *Saccharomyces cerevisiae* (MTCC 170) but the Ethyl acetate and chloroform extract from flower and root part demonstrated inhibitory effect against *Candida albicans* (MTCC 227), *Penicillium chrysogenum* (MTCC 160), *Candida albicans* (MTCC 3017), *Candida glabrata* (MTCC 3019), *Candida tropicalis* (MTCC 184) and *Saccharomyces cerevisiae* (MTCC 170) with zone of inhibition ranging from 8 to 10 mm except inactivity of flower chloroform extract on *Candida tropicalis*.

DISCUSSION

Search for new antimicrobial compound is very essential, as day by day resistance of the bacterial and fungal pathogens are showing increasing trends towards current antimicrobials. In recent years, a significant worldwide increase in fungal infections has been reported in the medico-logical case reports/ case studies. Fungi cause both superficial and internal mycoses. *Candida* spp. is an important cause of bloodstream infections and opportunistic infections in the oral cavity of immuno-compromised patients (Pfaller and Diekema, 2002). *Candida* species is also the most common cause of vaginal candidiasis or thrush and approximately 80-90% of thrush cases are caused by *Candida albicans* with other species (Hammer *et al.* 1998). The emergence of non-albicans *Candida* (NAC) species such as *Candida glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* which cause serious oropharyngeal candidiasis and occasionally esophageal candidiasis (Wingard, 1995; Vazquez *et al*., 2000) has also complicated the situation. Traditionally, *Tridax procumbens* has been used in medicine and to increase shelf life of foods, showing inhibition against bacteria, fungi and yeast. Most of their properties are due to the phytochemicals produced during their secondary metabolism (Adam *et al.,* 1998). As per the data obtained from the antifungal activity, *Candida* species, like *Candida albicans*, *Candida tropicalis* and *Candida glabrata,* and also the *Saccharomyces cerevisiae* were found to be the most susceptible to the inhibitory activity of tested extracts and were inhibited by 13 – 16 different extracts (Fig. 1 and 2).





The less affected fungi were *Rhizopus oryzae, Aspergillus niger, Aspergillus flavus* and *Penicillium chrysogenum*. Jindal and Kumar (2013a) studied the antimicrobial potential of flavonoids from *Tridax procumbens* against *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Trichophyton spp* and reported higher sensitivity of *Candida albicans* to flavonoids, indicating the antifungal potential of flavonoids from the plant. In present study also *Candida albicans* was found to the most sensitive fungus inhibited by sixteen different extracts prepared from four different parts of *Tridax procumbens.* On the other hand *Aspergillus niger* and *Aspergillus flavus* were found to be the least affected fungi. Essential oil extracted from *Tridax procumbens* have been reported to have antifungal activity (12 – 15 mm zone of inhibition) against the *Candida albicans, Candida tropicalis* and *Candida parapsilosis* (Manjamalai *et al.,* 2012). Results of present study also support the fact that extracts prepared from different parts of *Tridax procumbens* had the antifungal activity for *Candida* yeast. The spectrum of antifungal potency of extracts obtained from *Tridax procumbens* reflects the presence of active phytochemical constituents responsible for its antifungal activity. The phytochemical screening revealed the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), cardiac glycosides, terpenoids, fumaric acid, β-sitosterol, saponins and tannins in *Tridax procumbens* (Tejaswini *et al.,* 2011; Kamble and Moon, 2015; Ikewunchi, 2009). The antifungal activity noted in crude extracts may be due to the presence of active components which are present in *T. procumbens*. In light of the fact that microorganism are developing increasing resistance to currently used drugs, present investigation is of great importance in pharmaceutical industries for preparing plant based antimicrobial drugs.

In the present investigations, *Penicillium chrysogenum* was the less affected fungi, inhibited with low level (8 mm inhibition zone) only by flower and root extract prepared in acetone, ethyl acetate and chloroform solvent, while *Aspergillus niger* and *Aspergillus flavus* was totally unaffected by any of the solvent extract. Jindal and Kumar (2013b) reported excellent antifungal potential for free flavonoid of stem and bound flavonoid of stem and flower of *Tridax procumbens* against *Aspergillus niger*. Recently the production of biogenic metallic nanoparticles (NP) with least harmful effects to both humans and environment is scaling up. Bhati-kushwaha and Malik (2014) assessed the antifungal activity of silver nanoparticles obtained from callus extract (stem and leaf) of *Tridax procumbens* L. against *Aspergillus niger* and *Aspergillus flavus* and reported considerable antimicrobial activity of biogenic nanoparticles against *Aspergillus niger* and *Aspergillus flavus*. Comparison studies of crude extracts and synthesized biogenic nanoparticles from them also revealed superior antimicrobial activity of nanoparticles than crude extracts alone. Therefore metal nanoparticle technology could be an effective and eco-friendly way to increase an antimicrobial potential of biological extracts.

In the present studies methanol extract prepared from leaf, stem, flower and root showed significant inhibitory activity against *Candida albicans* (MTCC 227 and MTCC 3017) with inhibition zone ranging from 8 mm to 13 mm at 100 mg/ml concentration of extract, while root part extracted in methanol exhibited antifungal activity against *Candida tropicalis* and *Candida glabrata*. Manjamalai *et al*. (2012) studied the antifungal, anti-inflammatory potential and GC – MS analysis for bioactive molecules of *Tridax procumbens L.* leaf and reported inhibitory effect of leaf methanol extract against fungal cultures of *Candida albicans* and *Candida tropicalis* at different concentration of 150, 250, and 500µg/ml. They also identified alpha (α) and beta (β) pinenes, Sabinene, and l-Phellandrene in essential oil extracted from *Tridax procumbens* L. which have tremendous medicinal value. Previously, β-pinenes from the leaves of *Sesuvium portulacastrum* have been reported to possess antifungal activity against *Candida* spp. (Magwa *et al.*, 2003). However, our findings also indicate the susceptibility of *Candida albicans* and *Candida tropicalis* toward the methanolic leaf extract of *Tridax procumbens* L., which support the evidence for presence of bioactive compounds in the methanolic root extracts and it could be the better and effective anti-candidial drug in future. Use of such natural fungicidal agents may be effective and less toxic than the commercial chemical fungicides available for the therapeutic management of *Candida* infections. The mechanisms that is responsible for the antifungal activity is thought to be because of the phytochemicals present in the plant that shows a greater inhibitory activity against microorganisms.

Conclusion

From the present investigation, it is concluded that the plant *Tridax procumbens* had the immense antifungal potential for the tested *Candida* species and the assayed extract composed of active secondary metabolites responsible for its antifungal activity. The study validated the use of this plant as an antifungal agent in the management of infections caused by the assayed fungi. The results also provides acceptance to the folkloric use of this plant in treating microbial infection and showed that *Tridax procumbens* could be exploited for new potent antimicrobial agents.

REFERENCES

Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M. 1998. Antifungal activities of *Origanum vulgare* subsp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem*., 46: 1739-1745.

Ali, M., Rawinder, E. and Ramachandran, R. 2001. A new flavonoid from the aerial parts of *Tridax procumbens*. *Fitoterapia*, 72(3): 313-315.

Bhati-kushwaha, H. and Malik, C.P. 2014. Assessment of antibacterial and antifungal activities of silver nanoparticles obtained from the callus extracts (stem and leaf) of *Tridax procumbens* L. *Ind. J. Biotechnol.*, 13: 114 – 120.

Hammer, K.A., Carson, C.F. and Riley, T.V. 1998. *In vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida* spp. *J. Antimicrob. Chemether*., 42 :591-595.

Hill, A.F. 1952. Economic Botany. A textbook of useful plants and plant products, 2 nd edn. McGraw-Hill Book Company Inc, New York.

Ikewunchi, J.C., Catherine, C., Ikewunchi and Ngozi gboh M.I. 2009. Chemical profile of *Tridax procumbens Linn*, *Pak. J. Nutri.*, 8(5): 548-550.

Jindal, A. and Kumar, P. 2013a. Antimicrobial potential of flavonoids from *Tridax procumbens* against the pathogenic microorganisms. *Int. Res. J. Pharm*., 4(2): 119-121.

Jindal, A. and Kumar, P. 2013b. *In vitro* antifungal potential of Tridax procumbens L. against *Aspergillus flavus* and *Aspergillus niger*. *Asian Journal of Pharmaceutical and Clinical Research*, 6(2): 123 – 125.

Kamble, V.A. and Moon, A.H. 2015. Phytochemical analysis and antibacterial activity of active extracts from *Tridax procumbens* L. against selected pathogens. *Ind. J. Appl. Res.*, 5(2): 671-674.

Magwa, M.L., Gundidza, M., Gweru, N., Humphrey, G. 2003. Chemical composition and biological activities of essential oil from the leaves of Sesuvium portulacastrum. *J. Ethnopharmacol*., 103(1): 85–89.

Manjamalai, A., Sathyajit Singh, S.R., Guruvayoorpuram, C. and Berline Grace, V.M. 2010. Phytoconstituent and microbial activity of some medicinal plant on Tamil Nadu India. *Global J. Biotech. Biochem*., 5(2): 120-128.

Manjamalai, A., Varghese, S.S., Haridas A. and Berlin Grace V.M. 2012. Antifungal, anti-inflammatory and GC – MS analysis for bioactive molecules of *Tridax procumbens* L. leaf. *Asian J Pharmaceut. and Clin. Res.*, 5(1): 139 – 145.

McNeil, M.M., Nash, S.L., Hajjeh, R.A., Phelan, M.A., Conn, L.A., Plikaytis, B.D. and Warnock, D.W. 2001. Trends in morta mycotic diseases in United States 1980-1997. *Clin. Infect. Dis.*, 33: 641-647.

Mundada, S. and Shivhare, R. 2010. Pharmacology of *Tridax procumbens* a Weed, Review. *Int. J. Pharm. Tech. Res.*, 2(2): 1391-1394.

Pfaller, M.A. and Diekema, D.J. 2002. Role of sentinel surveillance of candidemia: Trends in species distribution and antifungal susceptibility. *J Clin. Microbiol*., 40: 3551-7.

Saraf, S., Pathak, A.K. and Dixit, V. K. 1991. Hair growth promoting activity of *Tridax procumbens* L. *Fitoterapia*., 62: 495-498.

Sharma, B. and Kumar, P. 2009. Extraction and Pharmacological Evaluation of Some Extracts of *Tridax procumbens* and *Capparis deciduas. Int. J. Appl. Res. in Nat. Prod.,* 1(4): 5-12.

Singh, V.K., Govil, J.N., Hashmi, S. and Singh, G. 2003. Ethnomedicine and pharmacognosy- II, Studium Press. USA. 7: pp 200.

Srinivasan, D., Sangeetha, N., Suresh, T. and Perumalsamy P.L. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J. Ethnopharmacol.*, 74: 217-220.

Tânia, da. S., Agostini-Costa, Roberto, F., Vieira, H.R., Bizzo, D.S., and Marcos, A.G. Secondary metabolites, chromatography and its applications, www.intechopen.com, Access on 22 Nov 2014.

Tejaswini, K., Vishwanath, P.B., Rudrama, D.K., Shylaja, S. and Jyothsna K. 2011. Phytochemical screening and antimicrobial activities of plant extract of *Tridax procumbens. The Bioscan*., 6 (2): 321-323.

Vazquez, J.A., Arganoza, M.T., Boikov, D., Vaishampayan, J.K. and Akins, R.A. 2000. *In vitro* susceptibilities of *Candida* and *Aspergillus* species to *Melaleuca alternifolia* (tea tree) oil. *Rev Iberoam Micol*., 17: 60-63.

Wingard, J.R. 1995. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin. Infect. Dis*., 20: 115-25.

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