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Anticandidal activity and phytochemical analysis of certain medicinal plants from Eastern Ghats, India

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The ethno-medico-botanical studies in Eastern Ghats provided an evidence for the tribal claims on the usage of medicinal plants for various human infectious diseases. The present investigation focused on the anticandidal activity of certain medicinal plants used for infectious diseases. The plant samples were extracted with solvents initially with petroleum ether followed by ethyl acetate and ethyl alcohol and the extracts were tested for anticandidal activity. The results indicated that, the extracts of twenty three medicinal plants significantly inhibited the growth of the test pathogen. The details of anticandidal activity, Minimum Inhibition Concentration (MIC) of active extracts and their phytochemical analysis with respect to alkaloids, flavonoids, glycosides, terpenes, saponins and essential oils were provided. The plants namely *Andrographis nallamalayana* Ellis*, Boswellia ovalifoliolata* Bal. et Henry*, Crotalaria madurensis* var. *kurnoolica* Ellis et Swaminathan*, Hedychium coronarium* Koeing*, Pterocarpus santalinus* L.f.*, Shorea tumbuggaia* Roxb.*, Tylophora fasciculata* Buch.-Ham. ex Wight were endemic and their extracts exhibited significant anticandidal activity.

Keywords: Anticandidal activity, Eastern Ghats, Ethno-medico-botanical studies, Phytochemical screening.

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Introduction

Candida albicans is an opportunistic pathogen of oral and genital infections in humans and a cause of morbidity and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). It is frequently found in the mouth and gastrointestinal tract without harmful effects, although its overgrowth results in to candidiasis in HIV-positive patients. *Candida* infections of the mouth, skin or vagina occur due to use of antibiotics that destroy beneficial as well as harmful microorganisms permitting *Candida* to multiply in their place resulting in *candidiasis* (moniliasis) or fungemia or a "yeast" infection[1,2].

C. albicans is emerging as major agent of hospital-acquired infections and they are ranked as the third most commonly isolated bloodstream pathogens, second most frequent isolate from blood cultures in hospitals with large population of immunocompromised patients[3]. *Candida* species are the fourth leading cause of nosocomial bloodstream infection in the United States, accounting for 8 to 10 % of all BSIs acquired in the hospital and the mortality rate of *Candida* BSI is 40 %, then 2,800 to 11,200 deaths each year may be associated with nosocomial candidemia. There have been several reports about drug resistance which becomes an important problem in a variety of infectious diseases including HIV infections, tuberculosis and other fungal or bacterial infections, that have profound effects on human health[4]. High degrees of anti-fungal drug resistance have been reported in *Candida* species which exhibited resistance towards available antibiotics namely, 5- Flucytosine (5-FU), Fluconazole and Ketaconazole[5]. In India, the occurrence of *Candida* in vaginal swabs of infertile women is 13 %, of which 40.7 % isolates are *C. albicans*[6]. About 90 % of AIDS patients experience at least one episode of Orophyrangeal candidiasis and up to 50 % develop oesophageal infection[7]. Although the capsules of Ketoconazole and Itraconazole are effective in the treatment of oral and oesophageal candidiasis in HIV-infected patients and their use in patients have been limited because of problems with drug absorption and development of azole-resistant *Candida* infections[8] besides side

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effects[9]. The rise in the incidence of fungal infections has exacerbated the need for next generation of antifungal agents as the available drugs have undesirable side effects and ineffective against reemerging fungi. Moreover, there is an urgent need for excavation of antifungal drugs that actually kill the fungus (fungicidal) rather than only inhibiting its growth (fungistatic).

One possible approach is to study the medicinal uses, phytochemical profile and screening of the drug agents for novel biological activities such as antibacterial and antifungal substances[10]. Furthermore, in the last few years, the number of immunosuppressed and immunocompromised patients, who frequently develop opportunistic systemic and superficial mycoses[11,12] such as candidiasis, dermato-mycosis, fungal infections etc., have increased dramatically[13,14]. The present investigation, carried for scientific validation of anticandidal activity of the medicinal plants from the study area, is based on the claims on effective use for the treatment of various diseases and sustainable utilization. The paper deals with the anticandidal activity of native medicinal plants and the screening has been performed in light of the above cited reasons. The present investigation pinpoints on the ethno-medico-botanical, anticandidal activity and phytochemical studies of 23 indigenous medicinal plants using in various human ailments hither to not reported. The results may provide scope for wide applications in medicinal chemistry and pharmacological evaluation to develop novel anticandidal drugs.

Materials and Methods

Study area

Eastern Ghats, one of the rich biodiversity areas of India, is harboring various semi nomadic tribes. The Eastern Ghats cover an area of about 75,000 km^2 traversing the Coromandel coast between 11° 30′ – 22° 00′ N latitudes and 76° 50′ – 86° 30′ E longitudes, showing discontinuous hill ranges with undulated topography. The Eastern Ghats of Andhra Pradesh state privileged with presence of rich floristic wealth and ornamented with tribal pockets, passes through Vizayanagaram, Srikakulam, Visakhapatnam, East and West Godavari, Krishna, Kurnool, Prakasam, Kadapa, Nellore and Chittoor districts[15]. There are 27 tribal communities confined to the isolated hills and adjacent plains of Eastern Ghats. The major tribes are *Bagatas*, *Chenchus*, *Jatapus*, *Khonds* (*Samantas*), *Konda doras*, *Konda kammaras*, *Konda reddis*, *Koyas*, *Lambadis* (*Sugalis*), *Nuka doras* (*Muka doras*), *Porjas* (*Gadabas*), *Savaras* and *Valmikis*[16].

Collection of plant materials

The extensive and intensive ethno-medico-botanical field explorations were conducted in the forests of Eastern Ghats and the information collected from bush/witch doctors, revealed 350 drug-yielding plants during 1998 to 2005[17-19]. The information regarding the drug-yielding plants was recorded following the standard methods[20-22] from the Adivasi tribes inhabited in the forested and rural areas of the Eastern Ghats of Andhra Pradesh.

The systematic enumeration of medicinal plants and the properties, viz. vernacular names, parts used, purposes, mode of administration, etc. were recorded in the field notebooks as well as audiotapes. The information was cross checked with other adivasi inhabitants in order to evaluate the authenticity of the drugs. Based on the folk evidences regarding the effective utilization for different human ailments the plant samples were collected and screened for anticandidal properties and phytochemical analysis.

Identification of plants

The voucher specimens were identified by the authors with the help of regional floras[23,24]. The plant species were collected during June and July of 1998 and 2005 and the same were deposited at Sri Krishnadevaraya University Herbarium, Anantapur.

Preparation of plant extracts

About 50 g of plant samples were shade dried, powdered and extracted initially with petroleum ether and successively with ethyl acetate and ethanol (250 mL) using a Soxhlet apparatus. The extracts obtained were filtered and concentrated separately under the reduced pressure, below 40 °C to dryness and used for anticandidal activity.

Preliminary phytochemical analysis

The active principles of many drugs found in plants are secondary metabolites[25,26]. Therefore, basic phytochemical investigation of these extracts for their major phytoconstituents is also vital. Hence, a preliminary phytochemical screening of the plants was conducted following the standard protocols[27,28]. In present investigation, maximum emphasis was given to alkaloids, flavonoids, glycosides, terpenes and volatile oils which are the major groups of compounds distributed widely and might be responsible for the various therapeutic properties.

Anticandidal susceptibility tests

Sterile Whatmann No.1 paper discs of 6 mm diameter were taken and soaked in the extracts containing 1 mg/mL final concentration for 24 h. The discs, impregnated with extracts were dried properly and used in the anticandidal activity. It was performed by employing the pour plate and disc diffusion methods[29]. The suspension cultures of *Candida albicans* MTCC181 obtained from IMTECH, Chandigarh, India, prepared in the nutrient broth, were inoculated in the nutrient agar in petri dishes at room temperature in sterile condition and mixed thoroughly to ensure uniform distribution. The discs containing drug extracts were placed carefully on the surface of the solidified nutrient agar seeded with 0.1 mL of suspensions (5 x 10^5 CFU/mL) of *C. albicans* and incubated for 32 h at 30 °C. Observation of clear zone around the paper disc was considered as positive result. Standard antibiotics namely kanamycin and fuconozole obtained from Hi-media, Mumbai, were used as positive controls. The inhibition zones formed around the discs were measured and expressed in millimeters. Three independent trials were conducted for each concentration and mean values were selected.

Anticandidal activity

The zone of inhibition surrounding the paper disc indicates anticandidal activity of the tested extracts was measured accurately to the nearest millimeter by means of metric ruler and illuminated colony counter. In all cases where the zone of inhibition was 10 mm ascertained whether fungistatic or fungicidal. The fungicidal activity was confirmed by transferring a loop of the culture from the inhibition zone transferred in to fresh sterilized nutrient broth and incubated under the standardized conditions. Simultaneously, standard antibiotics were tested for anticandidal activity in similar conditions so as to compare the degree of inhibition exhibited by the extracts. The extracts subjected to the test of susceptibility were found free of microorganisms. Each plate carrying a disc with DMSO and solvents alone served as negative control.

Minimum inhibitory concentration (MIC)

The MIC was determined using a broth micro dilution method[30]. Serial doubling dilutions of the extracts were prepared in a 96-well micro titer plates ranging from 0.05 to 200 mg/mL. For crude extracts the samples were dissolved in DMSO to create stock solutions (10 mg/mL). Two fold dilutions of each extract were carried out starting from 0.07 to 5 mg/mL. Aliquots of 10 µL of the previously prepared suspension of *C. albicans* (5 x 10^5 CFU/mL) were added to each well. The plates were incubated for 18 h at 30 ^oC and then were examined with Elisa reader (TECAN, Sunrise, China) at 620 nm and the lowest concentration of each extract showing no visible growth was taken as its MIC. The solution DMSO (100 µL/mL) served as the negative control. All the samples were tested in triplicates to confirm the activity.

Results and Discussion

The paper describes the ethnobotanical inventory, phytochemical screening and anticandidal activity of selected medicinal plants. The results of the biological activity revealed that the selected 23 medicinal plants listed (Table 1) showed significant anticandidal activity along with the minimum inhibition concentrations (Table 2). Among the test plants *Andrographis nallamalayana, Boswellia ovalifoliolata, Crotalaria madurensis* var. *kurnoolica, Curcuma neilgherrensis, Cycas beddomei, Hedychium coronarium, Moringa concanensis, Pterocarpus santalinus, Shorea tumbuggaia, Syzygium alternifolium, Terminalia pallida, Tylophora fasciculata* were endemic[31] medicinal plants of the study area which exhibited significant inhibitory activity on the test pathogen. Some species namely *Acalypha alnifolia, Vitex altissima, Acanthospermum hispidum, Canthium dicoccum, Cipadessa baccifera, Zingiber roseum, Vitex altissima* (Table 1) were rare and played vital role in inhibiting the growth of the *C. albicans* (Table 2). Anticandidal activity of *B. ovalifoliolata* leaf extracts was reported by Ratnam and Raju[32]. Essential oil extracted from *Piper hymenophyllum* fruits showed good inhibition against *C. albicans*[33]. Leaf and fruit extracts or essential oils of *S. alternifolium* strongly suppressed the growth of *C. albicans*[34,35]. Further, phytochemical screening of the selected medicinal plant extracts showed positive results to the chemical compounds like alkaloids, flavonoids, glycosides, terpenes, saponins and volatile oils (Table 3).

Statistical analysis of phytochemical screening revealed that flavonoids and glycosides (each 10 spp) were dominant compounds and other chemical constituents like volatile oils (8 spp), terpenoids (6 spp) and saponins (5 spp) present in lower

Table1—Medico-botanical enumeration of crude drugs used for anticandidal activity

S. No.	Botanical Name, Voucher No	Family	Vernacular name (Telugu)	Mode of treatment
1.	*Acalypha alnifolia* Klein ex Willd, 27040	Euphorbiaceae	*Mirapakuppinta*	Leaf extract given orally for dysentery
2.	*Acanthospermum hispidum* DC., 20508	Asteraceae	*Seemapalleru*	Whole plant ground, juice applied as an external application for Skin diseases.
3.	*Ammannia baccifera* L., 23833	Lythraceae	*Agnivendramu*	Plant paste mixed in goat milk and given orally and paste applied on wound.
4.	*Andrographis nallamalayana* Ellis, 26927	Acanthaceae	*Kachugadda*	Root mixed with a pinch of lime and chewed for mouth ulcers
5.	*Boswellia ovalifoliolata* Bal. et Henry, 27703	Burseracece	*Konda sambrani*	Gum dissolved in water and given orally for ulcers.
6.	*Canthium dicoccum* (Gaertn) Merr., 20418	Rubicaeae	*Nallabalusu*	Wood made into paste, used in head bath for Dandruff.
7.	*Cipadessa baccifera* (Roth.) Miq., 24119	Meliaceae	*Adavikarivepa*	Fresh leaves ground, mixed with sesame oil and applied externally in Skin diseases
8.	*Crotalaria madhurensis* var. *kurnoolica* Ellis et Swaminathan, 26967	Fabaceae	*Adavijanumu*	Fresh leaves crushed and paste applied externally on scabies.
9.	*Cycas beddomei* Dyer, 8669	Cycadaceae	*Peritha*	Leaf paste applied for scabies
10.	*Euphorbia fusiformis* Buch.-Ham. ex. D.Don, 27030	Euphorbiaceae	*Palasepu gaddalu*	Root tubers, pepper and garlic ground and the paste given orally for fever.
11.	*Globba marantina* L., 20655	Zingiberaceae	*Kalingadumpa*	Fresh rhizomes crushed, mixed with *Pongamia* seed oil and paste applied on white spots.
12.	*Gmelina asiatica* L., 20699	Verbinaceae	*Gummudu*	Fruit pulp as an external application in eczema.
13.	*Hedychium coronarium* Koeing, 27019	Zingiberaceae	*Konda allam*	Decoction of rhizomes given orally for rheumatism.
14.	*Piper hymenophyllum* Miq., 24200	Piperaceae	*Adavi tamalapaku*	Leaves mixed with turmeric and ground in gingelly oil, paste applied externally for mouth ulcers.
15.	*Premna latifolia* Roxb., 23836	Verbinaceae	*Nallichettu*	Stem bark ground with pepper and infusion given orally in the early mornings for ringworm.
16.	*Pterocarpus santalinus* L.f., 18940	Fabaceae	*Rakhta chandanamu*	The stem bark soaked in water for 8 hours and the infusion given orally.
17.	*Sapium insigne* (Royle) Benth., 6650	Euphorbiaceae	*Deva surapi*	Mixed with rice powder, made into balls and given orally as purgative.
18.	*Shorea tumbuggaia* Roxb., 24110	Dipterocarpaceae	*Tahmbajalari*	Stem bark extract given orally for ulcers.
19.	*Syzygium alternifolium* (Wt) Walp., 27708	Myrtaceae	*Mogi*	Infusion of seeds given orally once a day for 40 days in diabetes. Fruit paste applied externally wounds
20.	*Terminalia pallida* Brandis, 24121	Combretaceae	*Tellakaraka*	Fruits dried, ground and paste applied on swellings inflammation
21.	*Tylophora fasciculata* Buch.-Ham. ex Wight, 27010	Asclepiadaceae	*Mukkupala teega*	Leaf paste applied externally on the wounds
22.	*Vitex altissima* L.f., 27017	Verbenaceae	*Nemali adugu*	Root extract given orally for snake bite. Stem bark ground, made into paste, applied externally.
23.	*Zingiber roseum* (Roxb.) Roscoe, 23814	Zingiberaceae	*Adaviallamu*	Rhizomes ground, made into fine paste and applied externally for skin diseases

Table 2—Anticandiadal activity of selected medicinal plant extracts

S. No.	Botanical Name	Part used	Zone of inhibition* (mm)			MIC (µg/mL)		
			PE	EoAc	EOH	PE	EoAc	EOH
1.	*Acalypha alnifolia* Klein ex Willd	WP	12	-	9	1250	-	1250
2.	*Acanthospermum hispidum* DC.	L	-	10	16	-	500	250
3.	*Ammannia baccifera* L.	L	-	-	12	-	-	1500
4.	*Andrographis nallamalayana* Ellis	L	-	9	8	-	1500	1500
5.	*Boswellia ovalifoliolata* Bal. et Henry	L	-	7	10	-	1250	625
6.	*Canthium dicoccum* (Gaertn) Merr.	L	-	-	10	-	-	1500
7.	*Cipadessa baccifera* (Roth.) Miq.	L	18	12	-	250	500	-
8.	*Crotalaria madhurensis* var. *kurnoolica* Ellis et Swaminathan	L	-	9	13	1250	1250	625
9.	*Cycas beddomei* Dyer	Sb	-	10	14	-	1000	1500
10.	*Euphorbia fusiformis* Buch.-Ham. ex. D.Don	Rt	10	-	12	1250	-	1250
11.	*Globba marantina* L.	Rh	16	12	-	500	250	-
12.	*Gmelina asiatica* L.	Fr	-	12	16	-	1500	750
13.	*Hedychium coronarium* Koeing	Rh	10	-	9	625	-	1250
14.	*Piper hymenophyllum* Miq.	L	-	12	8		312	625
15.	*Premna latifolia* Roxb.	L	12	10	-	1000	1500	-
16.	*Pterocarpus santalinus* L.f.	Sb	-	-	10	-	-	1250
17.	*Sapium insigne* (Royle) Benth.	Sb	9	-	10	1250	-	625
18.	*Shorea tumbuggaia* Roxb.	Sb	-	11	7	-	312	625
19.	*Syzygium alternifolium* (Wt) Walp.	L	8	-	-	1500	-	-
20.	*Terminalia pallida* Brandis	Fr	-	-	16	-	-	312
21.	*Tylophora fasciculata* Buch.-Ham. ex Wight	Wp	-	12	12	-	312	312
22.	*Vitex altissima* L.f.	L	-	9	13	1250	1250	625
23.	*Zingiber roseum* (Roxb.) Roscoe	Sb	-	-	12	-	-	1000
	STANDARD ANTIBIOTICS							
	Kanamycin[a]		20					500
	Fuconozole[a]		18					500

a-Kanamycin, EoAc-Ethyl acetate extract, EOH-Ethyl alcohol extract, Fr-Fruit, L-Leaf, MIC-Minimum inhibition concentration, PE-Petroleum ether extract, R-Root, Rh-Rhizome, Rt-Root tuber, Sb-Stem bark, Sd-Seed, Wp-Whole plant, *exhibited by the plant extracts at 1 mg/mL concentration

Table 3—Qualitative phytochemical screening of selected medicinal plants.

S.No.	Botanical Name	Part used	Alk	Flv	Glyc	Terp	Sap	VO
1.	*Acalypha alnifolia* Klein ex Willd	Wp	+	++	-	+	T	+
2.	*Acanthospermum hispidum* DC.	Wp	+	++	+	+	-	T
3.	*Ammannia baccifera* L.	St	-	-	++	T	-	T
4.	*Andrographis nallamalayana* Ellis	L	+	+	-	+	-	-
5.	*Boswellia ovalifoliolata* Bal. et Henry	L	++	++	+	-	+	+
6.	*Canthium dicoccum* (Gaertn) Merr.	W	+	++	++	+	++	T
7.	*Cipadessa baccifera* (Roth.) Miq.	L	+	+	++	++	T	++
8.	*Crotalaria madhurensis* var. *kurnoolica* Ellis et Swaminathan	Fl	+	+	-	-	-	++
9.	*Cycas beddomei* Dyer	L	-	+	++	+	T	-
10.	*Euphorbia fusiformis* Buch.-Ham. ex. D.Don	Sb	-	++	++	+	+	-
11.	*Globba marantina* L.	Rt	T	+	-	+	+	+
12.	*Gmelina asiatica* L.	Rh	+	+	++	++	-	++
13.	*Hedychium coronarium* Koeing	Fr	++	+	++	+	+++	+
14.	*Piper hymenophyllum* Miq.	Rh	+	+	-	+	-	++
15.	*Premna latifolia* Roxb.	Fr	+	+	-	-	-	++
16.	*Pterocarpus santalinus* Linn. f.	L	-	+	++	+	+	+
17.	*Sapium insigne* (Royle) Benth.	Sb	++	+	+	+++	-	-
18.	*Shorea tumbuggaia* Roxb.	Sb	++	+	++	+	+	-
19.	*Syzygium alternifolium* (Wt) Walp.	Sb	+	++	+	++	++	-
20.	*Terminalia pallida* Brandis	Wp	+	++	+	++	+	++
21.	*Tylophora fasciculata* Buch.-Ham. ex Wight	L	+	++	+	-	+	++
22.	*Vitex altissima* L.f.	Fr	-	++	++	T	++	-
23.	*Zingiber roseum* (Roxb.) Roscoe	Ap	+	+	+	-	T	-

Alk-Alkaloids, Fr-Fruit, Fvs-Flavonoids, L-Leaf, R-Root, Rh-Rhizome, Rt-Root tuber, Sb-Stem bark, Sap-Saponins, Sd-Seed, T-Trace, Terp-Terpenoids, VO-Volatile oils, Wp-Who1e plant, +-Present, --No reaction

concentration. These compounds might be responsible for the anticandidal activity. Of the 23 medicinal plants, four plant species i.e. *C. dicoccum*, *C. baccifera*, *Gmelina asiatica* and *Sphaeranthus indicus* showed positive results to maximum compounds. Variations in anticandidal activity of the test plants related to differences in the contents of active compounds. Most of the medicinal plants in the present study exhibited similar activity against Kanamycin, a standard antibiotic used in the study inferring that the plant extracts used in the investigation are potential sources of natural substances of antibiotics.

The results obtained indicated the existence of anticandidal activity showing rich quantity of secondary metabolites extracts. A good correlation was observed between the reported claims of these plants in traditional medicine against infectious diseases. The folk claims on the usage of the plants for anticandidal infections were substantiated by the data obtained from the present study.

Conclusion

The present investigation on antifungal activity of the medicinal plant extracts on *C. albicans* suggests that several plants have the potential to generate novel metabolites. The fractions which exhibited anticandidal activity may lead to the discovery of novel anticandidal agents with potential resistance to *C. albicans*, which pose serious threat to society. Overall, the study transpired that the bioactive constituents produced by medicinal plants are a rich source of novel metabolites exhibiting a wide range of anticandidal activity that could serve as selective agents for the maintenance of health. So the medicinal plants of Eastern Ghats of India have potential which could be exploited for a wide variety of therapeutic principles. The present investigation provides new vistas for further isolation, chemical characterization and toxicological studies of bioactive compounds which are under progress in the laboratory. In conclusion, the anticandidal activity of the plant extracts might be due to different types of active principles acting either individually or synergistically each with a single or a diverse range of biological activities which needs to be studied in detail.

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