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Full Length Research Paper

Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms

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Eleven plants were selected to evaluate their antioxidant and antimicrobial potentiality against some skin diseases caused by some bacteria and fungi. The dry powder was extracted in methanol by cold percolation method. Free radical scavenging activities like diphenylpicrylhydrazyl (DPPH), superoxide anion (O_2^-) scavenging activity, hydroxyl radical (·OH) scavenging activity, reducing capacity assessment and total phenol content were measured. Antimicrobial activity was done against 8 microorganisms by agar well diffusion method. All the plants exhibited a strong correlation between antioxidant activity and total phenol content. *Euphorbia hirta* exhibited best antioxidant activity. The plant extracts showed more activity against Gram positive bacteria and fungi. The best antimicrobial activity was shown by *Euphorbia tirucalli*. The study supports the folkloric use of *E. hirta* and *E. tirucalli* against some skin diseases caused by oxidative stress or by microorganisms.

Key words: Skin diseases, antioxidant activity, antimicrobial activity, medicinal plants.

INTRODUCTION

Infectious diseases, particularly skin and mucosal infections, are common in most of the tribal inhabitants due to lack of sanitation, potable water and awareness of hygienic food habits (Caceres et al., 1993; Desta, 1993). It has been estimated that skin diseases account for 34% of all occupational diseases (Spiewak, 2000). The skin serves many functions particularly: protection, thermo-regulation, percutaneaous absorption, secretory and sensory. As the primary interface between the body and external environment, the skin provides the first line of defense against broad injury by microbial and chemical agents. Many more factors other than trauma and primary skin disease have been identified as contributory to skin infections and these include immune deficiency disease, diabetes mellitus, supervenous discoloration, mucosal ulcers, alopecia and systemic or topical use of steroids (Jawetz et al., 1978; Vasudevan et al., 2009). The most damaging consequence of disruption to the skin is invasion by pathogenic microorganisms (Robert and Kupper, 1999). Skin diseases can be caused by a variety of microbes and the skin is a haven for many microbes.

The skin is exposed to a broad variety of biological, chemical and physical attacks. Among them is solar ultraviolet (UV) radiation and following UV exposure, reactive oxygen species (ROS) are produced and are believed to be largely responsible for skin damage, which includes erythema, photoageing and cancer (Motamed and Naghibi, 2010). Some of the mentioned deleterious effects are through interaction with proteins, lipids and DNA (Nishigori et al., 2003; Matsumura and Ananthaswamy, 2004; Wang et al., 2010). A number of skin diseases are believed to be associated with oxidative stress, including psoriasis, acne and coetaneous vasculitis (Myachi, 1993). There is also evidence that ROS are involved in allergic and irritant contact dermatitis. It is consented that ROS are deleterious to wound healing process due to harmful effects on cells and tissues (Umachigi et al. 2007).

The search for newer source of antibiotics is a global

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Abbreviations: UV, Ultraviolet; **ROS,** reactive oxygen species; **EDTA,** ethylenediaminetetraacetic acid; **PMS,** phenazine methosulphate; **NADH,** nicotinamide adenine dinucleotide; **NBT,** nitroblue tetrazolium.

challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Problem of resistance and environmental degradation and pollution associated with irrational use of orthodox medicines have necessitated renewed interest in nature as source of effective and safer alternatives in the management of human infections (Chah et al., 2006; Shinwari et al., 2009; Shinwari, 2010). Plants are used in the treatment of the both type of skin diseases caused by microorganisms and oxidative stress (Njoroge and Bussmann, 2007) and oxidative stress (Afaq and Mukhtar, 2006).

Some plants like *Curcuma longa*, *Curcuma amada*, *Euphorbia hirta*, *Euphorbia tirucalli*, *Haliotropium indicum* and *Pithecellobium dulce* have been used traditionally for the treatment of various skin diseases. To validate their ethnotherapeutic claim in skin and some other diseases, *Asteracantha longifolia, Daemia extensa, Euphorbia nerrifolia, Morus alba* and *Trichedesma indicum*, were evaluated for antioxidant and antimicrobial activity.

MATERIALS AND METHODS

Collection of plant materials

Fresh parts of 9 different medicinal plants were collected in the month of August/September, 2008, Rajkot, Gujarat and fresh rhizomes of 2 plants (*C. longa* and *C. amada*) were purchased from local market. The plants were identified at the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The plants were thoroughly washed with tap water, shade dried, crushed in a homogenizer to fine powder and stored in air tight bottles.

Extraction of plant materials

The powder of different plant parts (10 g) was defatted with petroleum ether (100 ml) and then extracted with methanol by cold percolation method (Parekh and Chanda, 2007a). The extraction flasks were kept on a rotary shaker for 24 h at 120 rpm. Thereafter, it was filtered through 8 layers of muslin cloth and was centrifuged at 5000 rpm for 15 min. The supernatant was concentrated to dryness under reduced pressure and stored at 4°C in air tight bottles.

Determination of total phenol content

The amount of total phenol content in the methanol extract of different plants was determined with Folin-Ciocalteu reagent method (McDonald et al., 2001). 0.5 ml sample (1 mg/ml) and 0.1 ml (0.5 N) Folin-Ciocalteu reagent was mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml saturated sodium carbonate was added and the resulting mixture was again incubated at room temperature for 30 min. The absorbance of the mixture was measured at 760 nm using spectrophotometer. Gallic acid was used as standard. The total phenol content is expressed in terms of gallic acid equivalent per gram of extracted compound.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The DPPH free radical scavenging activity of methanolic extract was studied using the method of McCune and Johns (2002) with some modifications. Various concentrations of the extracts in methanol (100 – 1000 µg) were added to DPPH solution in methanol (0.3 mM). The mixture was shaken vigorously and allowed to stand for 10 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm. Ascorbic acid was used as standard. Reactions were carried out in triplicate. The radical scavenging activity was expressed in terms of the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50% (IC_{50}).

Hydroxyl radical (·OH) scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and methanolic extract for hydroxyl radical generated by Fe^{3+}-ascorbate–ethylenediamine-tetraacetic acid (EDTA)–H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao (1990). The reaction mixture contained, in a final volume of 1.0 ml, 100 µl of 2-deoxy-2-ribose (28 mM in KH_2PO_4-KOH buffer, 20 mM, pH 7.4), 500 µl of the various concentrations of methanolic extract of different plants (250 to 1000 µg) in water, 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1: 1 v/v), 100 µl of 1.0 mM H_2O_2 and 100 µl of 1.0 mM ascorbic acid was incubated at 37°C for 1 h. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added to the test tubes and were incubated at 100°C for 20 min. After cooling, absorbance was measured at 532 nm against control containing deoxyribose and water. Ascorbic acid was used as a standard. Reactions were carried out in triplicate. The percentage inhibition was determined by comparing the results of the extracts and standard.

Superoxide anion (O_2^-) radical scavenging activity

Measurement for the superoxide anion scavenging activity of methanolic extract of all plants was carried out according to the method of Robak and Griglewski (1988) with some modifications. Superoxide radicals were generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by NADH oxidation and assayed by nitroblue tetrazolium (NBT) reduction. In this experiment, the superoxide radical were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (300 µM) solution, 0.5 ml NADH (936 µM) solution and 0.5 ml of different concentrations of methanolic extract. The reaction was started by adding 0.5 ml of PMS solution (120 µM) to the mixture. The reaction mixture was incubated at 25°C for 5 min and the absorbance was measured at 560 nm against blank sample using spectrophotometer. Gallic acid was used as standard.

Reducing capacity assessment

The reducing capacity of methanol extract of all 11 plants studied was determined according to the method of Athukorala et al. (2006) with some modifications. One milliliter of extract (100 - 500 ug) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 30 mM). The mixture was incubated at 50°C for 20 min. Then, 2.5 ml trichloroacetic acid (TCA) (0.6 M) was added to the mixture, which was then centrifuged for 10 min at 3000 x g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 6 mM) and the absorbance was measured at 700 nm in a spectrophotometer. Ascorbic acid was used as standard.

Table 1. Extractive yield (%) and total phenolic content of methanol extract of different plants.

No.	Plants	Family	Parts used	Extractive yield (%)	Total phenol content (mg/g)
1	*Asteracantha longifolia* Nees.	Acanthaceae	Aerial	8.15	73.16 ± 1.41
2	*Daemia extensa* R. Br.	Asclepiadaceae	Aerial	10.86	28.35 ± 0.91
3	*Euphorbia hirta* L.	Euphorbiaceae	Aerial	7.25	101.01 ± 0.70
4	*Euphorbia tirucalli* L.	Euphorbiaceae	Aerial	5.30	61.04 ± 1.95
5	*Euphorbia nerrifolia* L.	Euphorbiaceae	Aerial	5.03	52.46 ± 1.79
6	*Haliotropium indicum* L.	Boraginaceae	Aerial	6.29	75.82 ± 2.34
7	*Morus alba* L.	Moraceae	Leaves	10.20	37.06 ± 1.32
8	*Pithecellobium dulce* (Roxb.) Benth.	Mimoseae	Aerial	8.60	91.79 ± 1.71
9	*Trichedesma indicum* R. Br.	Boraginaceae	Aerial	4.15	66.52 ± 4.06
10a	*Curcuma amada* L.	Zingiberaceae	Rhizome	6.04	19.82 ± 0.07
10b	*Curcuma amada* L.	Zingiberaceae	Peels	5.11	21.62 ± 0.40
11a	*Curcuma longa* L.	Zingiberaceae	Rhizome	8.06	32.88 ± 0.29
11b	*Curcuma longa* L.	Zingiberaceae	Peels	4.75	41.73 ± 3.02

Antimicrobial susceptibility test

Microorganisms

The microbial strains are identified strains and were obtained from National Chemical Laboratory (NCL), Pune, India. Two Gram positive bacteria (*Staphylococcus aureus* ATCC25923 and *Bacillus subtilis* ATCC6633), two Gram negative bacteria (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* NCIM2719) and four fungi (*Candida albicans* ATCC2091, *Candida tropicalis* ATCC4563, *Candida neoformans* NCIM3542 and *Cryptococcus leuteolus* NCIM 3238) were studied for antimicrobial activity.

Antimicrobial assay

The test organism was activated by inoculating a loop full of the strain in 25 ml of Nutrient broth/Sabaroud´s Dextrose broth and kept overnight on a rotary shaker. Mueller Hinton agar and Sabaroud´s Dextrose agar medium were used, respectively, for antibacterial and antifungal susceptible testing. The assay was performed by agar well diffusion method (Perez et al., 1990; Parekh and Chanda, 2007b). 200µl inoculum (1×10^8 cfu/ml) was introduced into molten Muller Hinton agar and poured into Petri-dishes when temperature reached 40 – 42°C. The media was solidified and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). 100 µl of test drug (25 mg/ml in DMSO) was introduced into the well and the plates were incubated at 37/28°C for 24 /48 h for bacteria and fungi, respectively. Dimethyl sulfoxide (DMSO) was taken as a control. The experiment was performed 3 times, under strict aseptic conditions. The microbial growth was determined by measuring the diameter of the zone of inhibition in mm.

RESULTS

Extractive yield

The extractive yield of different plant extracts studied is given in Table 1. The extractive yield varied amongst the different plants. Maximum extractive yield was shown by *D. extensa* (10.86%), while minimum was in *T. indicum* (4.15%).

Total phenol content

The total phenol content of the methanolic extract of the selected plants is presented in Table 1. The results are expressed in terms of gallic acid equivalent per g of extracted compound. The methanolic extract of *E. hirta* presented highest phenolic content as compared to other plants followed by *P. dulce* and minimum was in *C. amada* rhizome and peel.

DPPH free radical scavenging activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The method involves determination of the decrease in the absorbance of DPPH radical at 517 nm. The methanolic extract of the selected plants and the standard compound exhibited a concentration dependent activity and their IC_{50} values are presented in Table 2. *E. hirta* exhibited a strong *in vitro* antiradical capacity whose IC_{50} value was 34 µg/ml which was quite near to that of standard ascorbic acid (IC_{50} = 11.4 µg/ml); it is followed by *C. longa* rhizome, *C. longa* peels and *M. alba* and *E. tirucallli*, respectively. The remaining plants had IC_{50} values more than 150 µg/ml. Only *D. extensa* did not show any activity (Table 2).

Hydroxyl free radical scavenging activity

The hydroxyl radical scavenging activity of the methanolic extract of the selected plants is presented in Table 2.

Only 3 plants (*E. hirta*, *E. tirucalli* and *C. amada* rhizome) showed hydroxyl radical scavenging activity. *E. hirta* and *E. tirucalli* showed a concentration effect however, *E. hirta* had considerably more activity than *E. tirucalli*. Their IC_{50} values were 480 and 760 µg/ml, respectively. The

Table 2. Antioxidant activity of methanol extract of different plants.

Plant	IC_{50} Values (µg/ml)		
	DPPH	Hydroxyl radical	Superoxide radical
A. longifolia	190	> 1000	> 1000
D. extensa	> 1000	> 1000	> 1000
E. hirta	34	480	224
E. tirucalli	123	760	900
E. nerrifolia	156	> 1000	> 1000
H indicum	175	> 1000	510
M. alba	85	> 1000	> 1000
P. dulce	526	> 1000	410
T. indicum	270	> 1000	> 1000
C. amada (Rhizome)	660	940	> 1000
C. amada (Peels)	230	> 1000	> 1000
C. longa (Rhizome)	60	> 1000	> 1000
C. longa (Peels)	66	> 1000	> 1000
Ascorbic acid	11.4	-	-
Gallic acid	-	140	185

hydroxyl radical scavenging activity of *C. amada* rhizome was quite less, having an IC_{50} value of 940 µg/ml.

Superoxide free radical scavenging activity

The superoxide free radical scavenging activity was shown by methanolic extract of four plants only. The results are presented in Table 2. In all the four plants, a concentration effect was observed. The maximum inhibition ability by methanolic extracts of *E. hirta*, *P. dulce*, *H. indicum* and *E. tirucalli* were 54.20% at 240 µg/ml, 53.71% at 500 µg/ml, 58.56% at 600 µg/ml and 50.70% at 1000 µg/ml, respectively, and IC_{50} values were 224, 410, 510 and 900 µg/ml, respectively. The remaining plants had IC_{50} values > 1000 µg/ml and *M. alba* did not show any activity.

Reducing capacity assessment

The reducing capacity of methanolic extract of selected plants and standard ascorbic acid increased with the concentration (Figure 1). The activity of *E. hirta* was found to be significantly more pronounced than the other plants (Figure 1c). It was followed by *P. dulce;* methanolic extracts of remaining plants exhibited very low level of reducing capacity.

Antimicrobial activity

Eleven different plants representing different families were included for evaluating their antimicrobial potential against two Gram positive bacteria (*S. aureus* and *B. subtilis*), two Gram negative bacteria (*E. coli* and *P. aeruginosa*) and four fungi (*C. albicans, C. neoformans, C. tropicalis* and *C. leuteolus*). The plants belonged to the families Acanthaceae, Asclepiadaceae, Boraginaceae, Euphorbiaceae, Fabaceae, Moraceae and Zingiberaceae.

The extract of selected plants exhibited variable degrees of antimicrobial activity against selected bacteria and fungi. Amongst 11 plants, *E. tirucalli* showed inhibition zone against all microorganisms studied. The large zone of inhibition was shown by *C. amada* peels against *B. subtilis*. All the plants extracts exhibited more or less similar activity against all the four fungi except *A. longifolia* and *T. indicum* which did not show any activity. All the plants extract showed more activity against Gram positive bacteria than Gram negative bacteria and fungal strain (Table 3).

DISCUSSION

Botanical antioxidants have been used successfully in the treatment and prevention of photo aging, as well as of contact dermatitis (Afaq and Mukhtar, 2006). Phenolics are considered to be the most effective inhibitors against contact dermatitis. Phenolic compounds may act by means of a non-specific mechanism, for example, anti-oxidant, but may also act via specific mechanisms, such as the inhibition of the mediators implicated in the immune response (Rios et al., 2005). It is important to examine the correlation between the content of total phenols and the antioxidant potential because some authors have

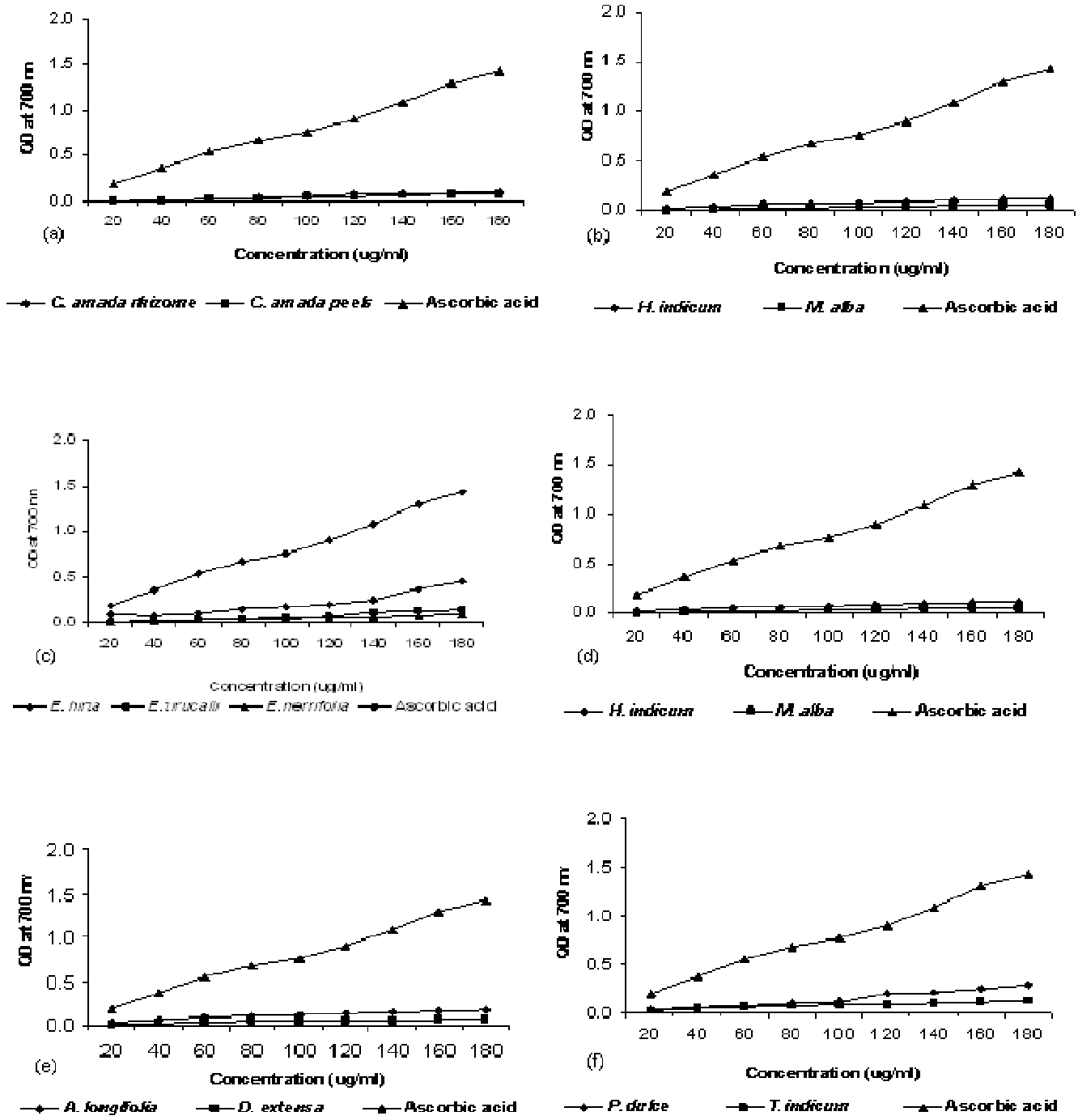

Figure 1. Reducing capacity assessment of methanol extract of different plants.

reported that there is no correlation between the content of antioxidant compound and the radical scavenging capacity (Yu et al., 2002). The results obtained in this study do not support these claims. In the present study, there is a strong correlation between total phenolic content and antioxidant activity. These data are in accordance with others who have shown that high total phenol content increases antioxidant activity (Velioglu et al., 1998; Holasova et al., 2002) and there is a linear correlation between phenolic content and antioxidant activity. Amongst the screened plants, *E. hirta* showed best hydroxyl radical scavenging activity (82%), superoxide free radical scavenging activity (IC_{50} = 224 µg/ml) and good reducing capacity.

Table 3. Antimicrobial activity of methanol extract of different plants.

Plant	Microorganisms							
	SA	BS	PA	EC	CT	CA	CN	CL
A. longifolia	11.5 ± 0.87	9.0 ± 0.00	9.0 ± 0.00	9.5 ± 0.29	-	-	-	-
D. extensa	-	13.0 ± 0.00	11.5 ± 0.29	11.0 ± 0.00	-	9.0 ± 0.29	-	-
E. hirta	12.0 ± 0.58	9.0 ± 0.00	12.0 ± 0.58	12.0 ± 0.58	-	-	10.5 ± 0.00	10.0 ± 0.00
E. tirucalli	11.0 ± 0.58	9.0 ± 0.00	14.0 ± 0.00	12.0 ± 0.58	9.5 ± 0.00	10.0 ± 0.29	10.5 ± 0.00	10.5 ± 0.29
E. nerrifolia	10.0 ± 0.00	9.5 ± 0.29	10.0 ± 0.00	12.0 ± 0.58	12.0 ± 0.29	-	-	-
H. indicum	10.0 ± 0.00	9.0 ± 0.00	10.0 ± 0.58	11.0 ± 0.58	10.0 ± 0.29	-	-	9.0 ± 0.00
M. alba	9.0 ± 0.00	-	9.5 ± 0.29	9.5 ± 0.29	12.5 ± 0.29	10.5 ± 0.00	-	10.0 ± 0.00
P. dulce	-	9.5 ± 0.29	9.5 ± 0.00	10.0 ± 0.29	10.0 ± 0.58	10.50 ± 0.00	9.5 ± 0.58	10.0 ± 0.00
T. indicum	13.0 ± 0.00	-	9.0 ± 0.00	10.0 ± 0.00	-	-	-	-
C. amada (Rhizome)	12.5 ± 0.29	14.0 ± 0.00	-	-	-	9.5 ± 0.00	-	10.5 ± 0.29
C. amada (Peels)	13.5 ± 0.29	16.0 ± 0.00	-	-	9.0 ± 0.29	10.0 ± 0.29	-	10.5 ± 0.29
C. longa (Rhizome)	10.5 ± 0.29	12.0 ± 0.00	-	-	10.5 ± 0.29	10.0 ± 0.29	10.7 ± 0.14	12.5 ± 0.29
C. longa (Peels)	10.5 ± 0.29	11.0 ± 0.00	-	-	10.0 ± 0.29	9.0 ± 0.29	9.5 ± 0.00	10.5 ± 0.29

Results are expressed as mean ± SEM. n = 3. SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, PA: *Pseudomonas aeruginosa*, EC: *Escherichia coli*, CT: *C. tropicalis*, CA: *Candida albicans*, CN: *Cryptococcus neoformans*, CL: *Cryptococcus leuteolus.*

The antibacterial activity of *C. longa* and *C. amada* were more pronounced on the Gram positive bacteria (*S. aureus and B. subtilis*) than the Gram negative bacteria. The reason for the difference in sensitivity between Gram positive and Gram negative might be ascribed to the differences in morphological constitutions between these microorganisms. The Gram negative bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components, which makes the cell wall impermeable to antimicrobial chemical substances. The Gram positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of Gram negative bacteria are more complex than Gram positive bacteria and thus, less susceptible to antimicrobial agents (Hodges, 2002; Babita et al., 2008; Costa et al., 2008). *E. tirucalli*, *M. alba* and *P. dulce* exhibited activity against all Gram positive bacteria, Gram negative bacteria and fungi thus, supporting folkloric use in the treatment of some skin diseases as broad-spectrum antimicrobial agents.

The observed antibacterial and antifungal effects on the bacteria and fungi may be due to the secondary metabolites present in them which have been shown to be responsible for antibacterial and antifungal properties (Cowan, 1999; Draughon, 2004). The mechanisms responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups through more nonspecific interactions with the proteins (Mason and Wasserman, 1987).

Some of the investigated plants (*D. extensa, E. nerrifolia, T. indicum, A. longifolia* and *H. indicum*) did not show strong antibacterial activity; however, negative results do not mean absence of bioactive constituents nor that the plant is inactive. The active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed and the strains used. Lack of activity can thus only be proven by using large doses (Farnsworth, 1993; Taylor et al., 2001).

Thus, from the overall results obtained, it is concluded that *C. amada*, *C. longa*, *P. dulce*, *E. tirucalli* and *M. alba* possess antimicrobial properties which act against some pathogenic organisms associated with skin infections.

The peels of *C. amada* and *C. longa* also possessed good antimicrobial activity indicating good utilization of a waste product. *E. hirta* possess antioxidant properties. They therefore justify their popular use by local herbalist against skin

diseases.

Conclusion

In conclusion, all the plants investigated possessed activity against at least one strain of bacteria and/or fungi. The extensive use of these herbal drugs by the local people in treating various types of skin disorders might therefore be justified by their antimicrobial activities against different strains of bacteria and fungi, which are common to be responsible for causing various skin diseases.

Also the results of this study support the use of *E. hirta* and *E. tirucalli* in traditional Indian medicine and show that methanolic extracts of these plants can be used as an easily accessible source of natural antioxidant and antimicrobial agent and can be of assistance in some dermatological problems.

The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitfulness results. Further studies might aim at the isolation and identification of active substances from the active plant extracts which could also disclose compounds with better therapeutic value.

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