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Research article

ANTIMICROBIAL ACTIVITY AND PHOTOCHEMICAL SCREENING OF *TINOSPORA CORDIFOLIA AND EUPHORBIA HIRTA*

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ABSTRACT: The present study deals with the antimicrobial activity and phytochemical screening of the two medicinal plants, *Tinospora cordifolia* and *Euphorbia hirta* those are commonly available in India. Results of antimicrobial activity revealed that these medicinal plant extracts were very effective against *Serratia marcescens, E. coli, Streptococcus thermophilus, Fusarium oxysporium, Aspergillus niger* while these extracts showed very less inhibition against Trichoderma reesei. Phytochemical analysis of these plants confirms the presence of various phytochemical like alkaloids, flavonoids, polyphenols, steroidal terpenes in Euphorbia hirta and alkaloids, flavonoids, Saponin, tannins, steroidal terpenes, reducing sugar in *Tinospora cordifolia*. While other phytochemical like, glycosides, phylobatamins, xanthoproteins, phenolic compounds were found to be absent in these extracts. These plants can be a source of useful drugs but further studies are required to isolate the active component from the crude plant extract for proper drug development.

Keywords: Phytochemical Screening, Antimicrobial assay, *Tinospora cordifolia* and *Euphorbia hirta*

INTRODUCTION

During the last decade, the use of traditional medicine has expanded globally and is gaining popularity as an alternative medicine. According to World health organization (WHO) more than 80% of the world population relies on traditional medicine for their primary health care needs (WHO, 2001). Plant synthesizes a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary & secondary metabolites. Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information be of value in disclosing new resources of such chemical substances. A variety of plants or materials derived from plants have been used for the prevention and treatment of diseases virtually in all cultures. Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed from one generation to another (Adedapo et al., 2005). Medicinal plants also represent a rich source from which antimicrobial agents can be obtained (Kubmarawa et al., 2007). Many pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine (WHO, 2008).

The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga et al., 2005), are termed as phytochemicals. Phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. They confer plants with odour (terpenoids), pigmentation (tannins and quinines), and flavor (capsacin) (Mallikharjuna et al., 2007) and are a part of plant naturally defense system. These bioactive components are said to be responsible for the antimicrobial effects of plant extracts *in vitro.* They are grouped as flavonoids, alkaloids, glycosides, Saponin, tannins, terpenoids, carbohydrates, and sterols. Plants have evolved a number of inducible defense mechanisms against pathogen attack. Some of the responses are constitutive and pathogen non-specific, but the majority of them are induced after recognition of the pathogen. Recognition results in the activation of a verity of defense responses, including rapid localized cell death (Hammond and Jones, 1996).

Tinospora cordifolia commonly known as Guduchi is a plant prescribed in Ayurveda, the Indian traditional system of Medicine as a Rasayana (Thatte and Dhanukar, 1986). It belongs to family Menispermaceae (Methew et al, 1999). Guduchi plant growing on Neem tree (Azadirachta indica) is bitterer and more efficacious and is said to incorporate the medicinal virtue of Neem also (Handa et al, 2008).

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The notable medicinal properties reported are anti-diabetic, anti-periodic, anti microbial, anti-inflammatory, anti-arthritic, antioxidant, anti-allergic, anti-stress, anti-leprotic, ant-malarial, hepatoprotective, immunomodulatory and antineoplastic activities (Krishna et al, 2009, Panchabhai et al, 2008, Upadyay et al, 2010, Ankita et al., 2012). Its root, steam and leaves, are used for their medicinal properties. The root of this plant is known for its anti-stress, anti-leprotic and anti-malarial activities. The anti-microbial activity of *T. cordifolia* was observed in root, stem and leaf extracts on pathogenic microorganisms (Jeychandran et al 2003, Mahesh et al, 2008, Samy et al, 2005). An impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine (Anjali et al, 2009).

The Euphorbia species have been used in the treatment of wounds in India ethno medicine (Ayyanar and Ignacimuthu, 2009). *Euphorbia hirta* L. is a medicinal, rhizomatous herb distributed in Southern Western Ghats of India and Northern East Coast of Tamil Nadu (Rahuman et al, 2007) The flowers are small, numerous and crowded together in dense cymes about 1 cm in diameter. The fruits are yellow, three-celled, hairy, keeled capsules, 1-2 mm in diameter, containing three brown, four-sided, angular, wrinkled seeds. The plant has been used for female disorders but is now more important in treating respiratory ailments, especially cough, bronchitis and asthma. In India it is used to treat worm infestations in children and for dysentery, gonorrhea, jaundice, pimples, digestive problems and tumors' (Kirtikal et al, 1991). The fresh milky latex is applied to wounds and warts and the root of the plant is used in sprains and inflammation, miscarriage, epilepsy, maggots in wounds and irregular growth of teeth (Jha et al, 1992). It is also used for coughs, chronic bronchitis and other pulmonary disorders in Malagasy. The plant is also widely used in Angola against diarrhea and dysentery, especially amoebic dysentery. In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Ogueke et al, 2007).

MATERIALS AND METHODS

Plant material:

The plants Tinospora cordifolia and euphorbia hirta were collected from the botanical garden of the Chandigarh College of Technology, Landran, Mohali, India.

Preparation of plant extracts

Water was used for extraction from the fresh leaves of test plant. Apparently plant leaves were collected washed thoroughly in tap water 3-5 times. And the leaves of the test plants were homogenized with 100ml of double distilled water followed by centrifugation at 10,000 rpm for 5 min to separate the liquid medium from debris or other particle. The supernatant was collected in falcon tubes and was stored below ambient temperature.

Test micro organisms for antimicrobial assay

Bacterial cultures- Serratia marcescens , E. coli (gram negative bacteria), Streptococcus thermophilus (gram positive bacteria) and Fungal cultures of *Candida albicans, Fusarium oxysporium, Trichoderma reesei, Aspergillus niger* were purchased from Microbial Type Culture Collection Centre (MTCC) at Institute Of Microbial Technology (IMTECH), Chandigarh, India and stored at -20ºC.

Preparation of inoculums

All the cultures were revived on selective media broth and were given the required incubation conditions specific of each culture. The gram positive (*Streptococcus thermophilus*) and gram negative bacteria (*Escherichia coli, Serratia marcescens*) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, The fungal inoculums (*Candida albicans, Fusarium oxysporium, Trichoderma reesei, Aspergillus niger*) was prepared from Potato dextrose agar medium.. Then these cultures were used for antimicrobial assay.

Procedure of Antimicrobial Assay

Antibacterial activity

For assaying antibacterial activity, the agar well diffusion method was used (Anonymous, 1996). About 20 ml of respective growth media was poured into the Petri plates. Once the agar got solidified, culture of bacteria was spread after mixing with small amount of GM broth. Four holes were made in the plates (about 5 mm diameter) using a sterile cork borer and equal volumes of the extracts were transferred into the holes using pipette. Two Petri dishes containing a particular micro-organism were used for each concentration of the extract. The plates were allowed to stand for one hour for prediffusion of the extract to occur and were incubated at 37 ± 2 ^{o}C for 24 hrs. At the end of incubation the plates were collected and zones of inhibition that developed were measured in mm.

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Antifungal Activity

The antifungal activity was tested by disc diffusion method [Taylor 1995]. The potato dextrose agar plates were inoculated with each fungal culture. The filter paper discs (5 mm diameter) impregnated with varying concentrations of plant extracts. Water was used to dissolve the extract and completely dried from discs before application on organism-seeded plates. The activity was determined after 72 h of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

Phytochemical screening

Flavonoids

To 1 ml of aqueous extract was added 1 ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids. (Njoku and Obi, 2009).

Saponin:

In 2 ml of test solution, 2N HCl was added in small amount and shaken. The aqueous layer was decanted and in this 2 drops of Mayer's reagent (potassium mercuric iodide) was added. The intense colour foaming lather was taken as positive test for Saponin.

Alkaloids

In 2 ml of test solution 10% NaOH solution was added and heated. The white turbidity and precipitate was taken as positive control for alkaloids.

Tannins

To 2 ml of test solution Con.HNO_3 was added along excess ammonia .the formation of white precipitate was taken as positive control for Tannins.

Carbohydrates (Molisch's test)

One drop of concentrated sulphuric acid was added to about 1g of the extract, and then three drops of 1% α-napthol in 80% ethanol were added to the mixture without mixing to form an upper phase. Formation of brown or purple ring at the interphase indicated the presence of carbohydrates (Abba et al., 2009).

Test for terpenoids (Salkowski test):

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was mixed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

RESULTS AND DISCUSSION

The results for antimicrobial activity of different plant extracts under study against bacteria are shown in Figure 1-4. The diameter of zone of inhibition decreased with concentration of plant extract. All the crude seeds extract had shown zone of inhibition against Serratia marcescens, Fusarium oxysporium, *Trichoderma reesei, Aspergillus niger, E. coli* at all concentrations and zone of inhibition decreased in size (mm) with concentration of crude seed extract. The crude seed extracts of both medicinal plants were ineffective to inhibit the activity of *Trichoderma reesei.*

This antibacterial potency may be due to the presence of many potent compounds such as flavonoids, terpenes, phenolics and alkaloids etc .The leaves extract of the plant species under study were found to contain Tannins, Cardiac glycosides, Terpenoids, Carbohydrates and Saponin. Phytochemicals are as antimicrobial compounds, have made great contribution for quick and effective management of plant disease and microbial contamination in several agricultural conditions. Phytochemical analysis of the two medicinal plants, *Tinospora cordifolia and Euphorbia hirta* is presented in Table 2. The leaves extract of the plant species under study were found to contain Tannins, Cardiac glycosides, Terpenoids, Carbohydrates and Saponin. Cardiac glycosides have anti-inflammatory activity (Shah et al., 2011), protect against lethal endotoxemia (Matsumori et al., 1997) and are used in cardiac treatment of congestive heart failure. Cichewicz & Thorpe (1996) have reported the membrane disruption and inhibitory effect of terpenoids against fungi and bacteria. Studies have shown that saponins have heamolytic property, induced cytotoxicity effect (Rao & Sung, 1995), expectorant action (Ayoola & Adeyeye, 2010), antitumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing (Nafiu et al., 2011).

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Saponins have the property of precipitating and coagulating red blood. These plants are used to stop bleeding and in treating wounds (Okwu & Josiah, 2006). They exhibit foaming properties and cell membrane- permeabilizing properties. Their soapy character is due to their surfactant properties (Noudeh *et al.,* 2010). Thus the secondary metabolites identified in the plant materials used in the study could be responsible for antimicrobial activity exhibited by the seeds extracts of the plants. Their varied occurrences in various plant extracts however indicate that probably, their therapeutic effect(s) are not the direct effect of a single group or compound, but rather that the compounds possibly act in combination to bring about an effect.

Table 1: Showing concentration of plant extract in each well

	Crude extract (µL)	Double distilled water (µL)
Well 1	100	0
Well 2	75	25
Well 3	50	50
Well 4	25	75

Table 2 Preliminary phytochemical results of extract of *Euphorbia hirta and Tinospora cordifolia:*

S. No.	Phytochemical present	*Result of Euphorbia hirta*	*Result of Tinospora cordifolia*
1	Alkaloid	++	++
2	Flavonoids	+	+
3	Saponin	-	+
4	Coumarins	+	+
5	Polyphenols	++	-
6	Cardiac glycosides	+	++
7	Triterpenes	+++	+++
8	Cyanogenic glycosides	-	-

(-) = negative result, (+) = small amount, (++) = average, (+++) = high amount

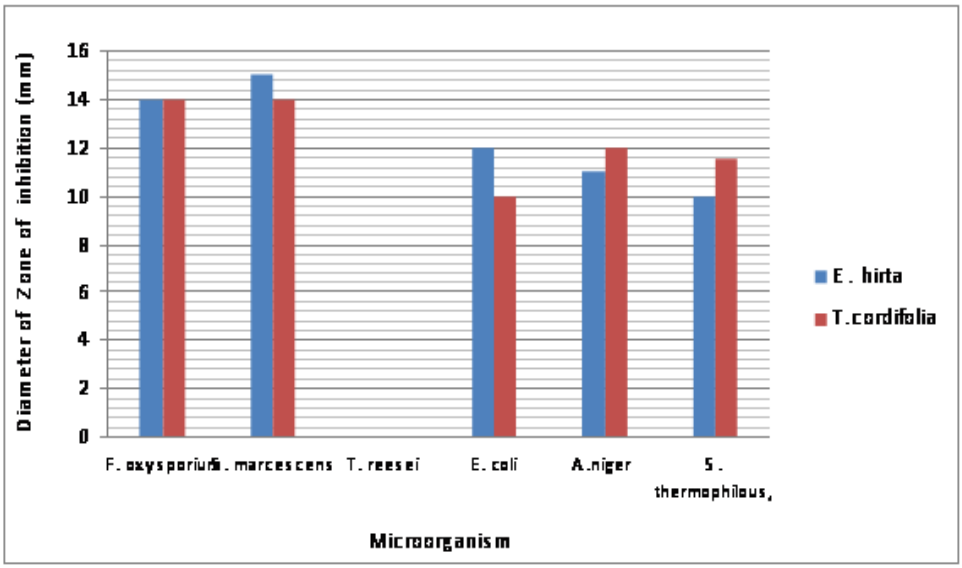

Figure 1 Graph showing the zone of inhibition against various micro organisms with 100% crude extracts of Euphorbia hirta and Tinospora cordifolia (well 1)

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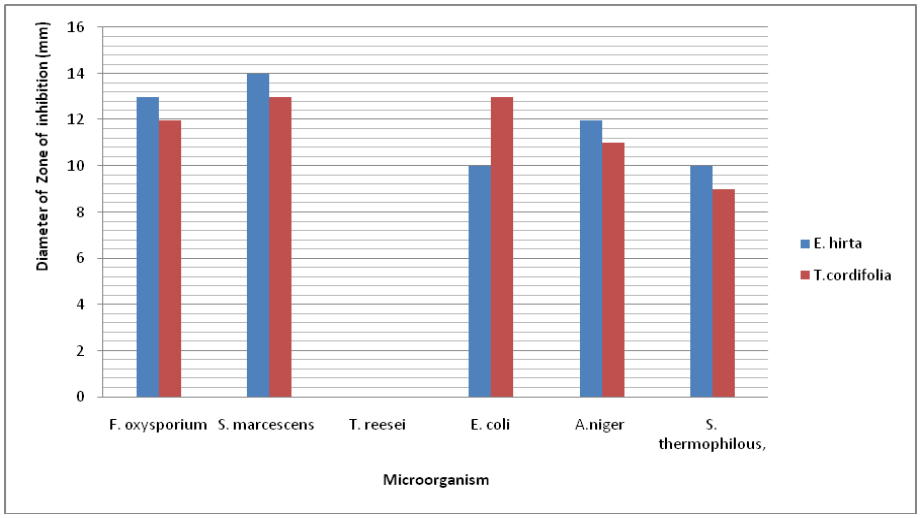

Figure 2 Graph showing the zone of inhibition against various micro organisms with 75% crude extracts of Euphorbia hirta and Tinospora cordifolia (well 2)

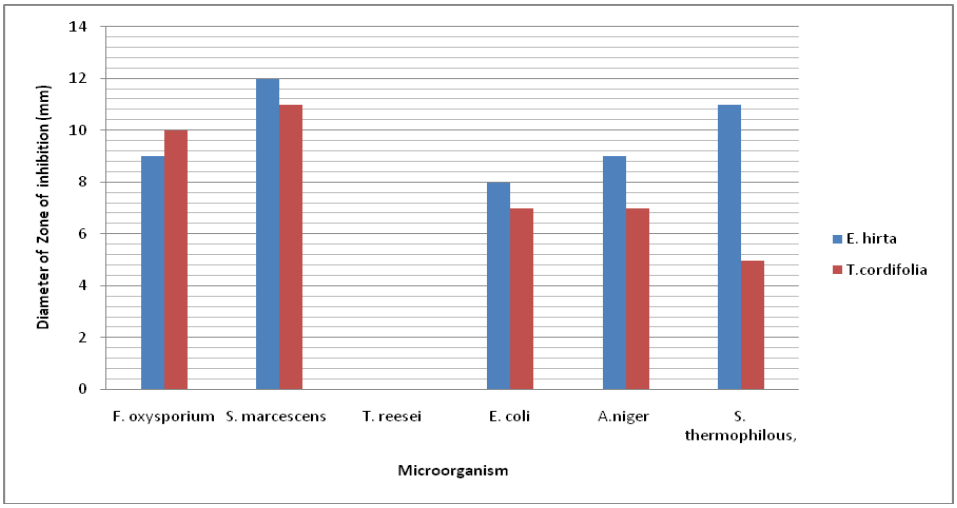

Figure 3 Graph showing the zone of inhibition against various micro organisms with 50% crude extracts of Euphorbia hirta and Tinospora cordifolia (well 3)

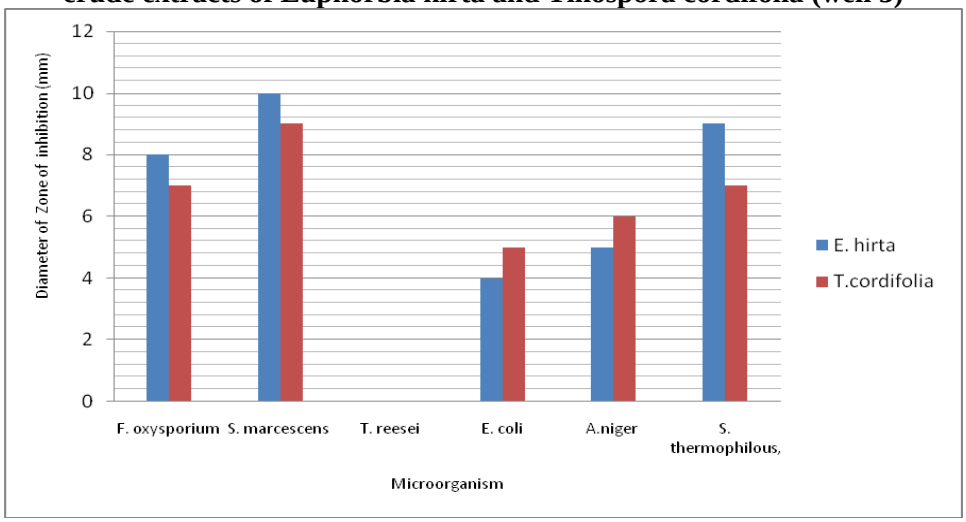

Figure 4 Graph showing the zone of inhibition against various micro organisms with 25% crude extracts of Euphorbia hirta and Tinospora cordifolia (well 4)

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CONCLUSION

The present study reveals that these plants under study can be used for the treatment of cancer, congestive heart failure, lowering of cholesterol levels in blood, healing of wounds, endotoxemia etc. since they contain various phytochemicals that are known to treat above mentioned diseases. The demonstration of broad spectrum of antibacterial activity by *Euphorbia hirta and Tinospora cordifolia* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease, chemotherapy and control. With the evidence of antibacterial and antifungal activities of the extracts of preparations under study, it can be rationally suggested that further work needs to be done to identify the chemical natures of the active principles as well as their modes of actions on bacterial cells and their roles in diseases curing. Further studies are needed with these plants under study to, characterize and elucidate the structure of the bioactive compounds of these plants for industrial drug formulation and to purify proteins from these plants which may act as a drug for the treatment of various diseases.

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