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Analysis of the Phytochemical Content and the Antibacterial, Antifungal and Antioxidant Activities of the Roots, Stems and Leaves of *Hemidesmus indicus*, *Ocimum sanctum* and *Tinospora cordifolia*

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Abstract: *Hemidesmus indicus* (*H. indicus*), *Ocimum sanctum* (*O. sanctum*) and *Tinospora cordifolia* (*T. cordifolia*) are three important medicinal plants in traditional Indian medicine. In this study, the methanolic and chloroform extracts of different plant parts (roots, stems and leaves) of *H. indicus*, *O. sanctum* and *T. cordifolia* were subjected to phytochemical, antibacterial, antifungal and antioxidant tests. Phytochemical screening of both extracts of each plant part revealed the presence of phytochemicals such as alkaloids, flavonoids, phenolic compounds, steroids and tannins. The methanolic extracts of all three plants exhibited greater antibacterial and antioxidant effects than the chloroform extracts. These effects could be related to the high content of phytochemical constituents such as alkaloids, steroids, tannins, flavonoids and phenols. When comparing the various plant parts, the roots of *H. indicus* had the highest number of antimicrobial activities, showing antibacterial properties against all of the investigated organisms except for one (*A. flavus*). The *H. indicus* leaf extract had antibacterial activities against *S. aureus*, *E. coli* and *K. pneumoniae* while the stem was effective against *P. vulgaris* and *A. niger*. The root of *T. cordifolia* has the second highest antimicrobial activity, acting against five different organisms (*B. subtilis*, *S. aureus*, *K. pneumoniae*, *P. vulgaris* and *A. niger*). *O. sanctum* leaf extract exhibits antibacterial activity against *B. subtilis* and *E. coli*, while the stem is only effective against *B. subtilis*. The methanolic root and stem extracts of *H. indicus*, the methanolic leaf extract of *O. sanctum* and the stem extract of *T. cordifolia* also have antioxidant potential.

Key words: *H. indicus*, *O. sanctum*, *T. cordifolia*, phytochemical analysis, antibacterial, antifungal, antioxidant activities

INTRODUCTION

Over the years, medicinal plants have been useful sources of several active compounds of recuperative value and have been used as a substitute medicine for treating numerous diseases (Nostro *et al.*, 2000). Exposure of microorganisms to various antibiotics have led to the development of resistance and has compelled researchers to scrutinize different natural sources to combat challenging strains (Gayathri and Kannabiran, 2009).

Hemidesmus indicus (*H. indicus*) belongs to the Asclepiadaceae family and is a renowned component in Ayurvedic and Unani medicines for treating various diseases, such as blood diseases, respiratory diseases,

biliousness, rheumatism, skin diseases, diarrhea, burning sensation, bronchitis, fever, antileukemic activity, eye diseases and gastric disorders (Austin, 2008). Due to the different biological properties and the extensive phytochemical studies of *H. indicus*, this plant has been included in the British Pharmacopoeia (Fimognari *et al.*, 2011) as a possible treatment for various diseases.

Because various parts of the plants have traditionally been used for different purposes, the activities of the different plant parts were investigated. *H. indicus* roots are reported to have potential inhibitory activity against viper venom toxicity (Alam *et al.*, 1994), antinociceptive activity and antioxidant activity (Ravishankara *et al.*, 2002). *H. indicus* leaf ethanolic extracts were documented

as antimosquitocidal agents and water purifiers. The ethanolic extracts of *H. indicus* leaves are believed to have wound healing activity and the methanolic extracts of *H. indicus* have hepatoprotective effects. The methanolic and aqueous extracts of *H. indicus* stem have antimutagenic and larvicidal effects (Khanna and Kannabiran, 2007).

Ocimum sanctum (*O. sanctum*) belongs to the *Limnaceae* family and is a common medicinal plant found in India. It is an important component in Ayurvedic treatments of various diseases. *O. sanctum* possesses several pharmacological properties such as antidiabetic (Khan *et al.*, 2010), antihypercholesterolemic, antioxidant (Gupta *et al.*, 2006), anti-stress, antinociceptive, anticancer, antifertility, cardioprotective, hepatoprotective, immunomodulatory, anti-inflammatory, anti-anaphylactic, mast cell stabilization and antihistaminic activities (Sridevi *et al.*, 2009). A decoction of *O. sanctum* root is used as a diaphoretic in malarial fever and genito-urinary disorders. *O. sanctum* seed oils have antioxidant, antidiabetic and antihypercholesterolemic effects. The aqueous decoction of *O. sanctum* leaves is used to treat patients with gastric and hepatic disorders (Prakash and Gupta, 2005). The leaves have been used to treat nausea and vomiting and as an anthelmintic agent, while the *O. sanctum* stem exhibits significant anticonvulsant activity (Jaggi *et al.*, 2003).

Tinospora cordifolia (*T. cordifolia*) belongs to the *Menispermaceae* family and is distributed all over India and in other parts of the world. This plant is valued for medicinal properties in Ayurvedic medicine. The whole plant possesses various activities such as antiulcer, hypolipidemic (Prince *et al.*, 1998) antipyretic, anticancer, antidiabetic, jaundice, immunomodulatory and hepatoprotective activities. The *T. Cordifolia* root has been clinically used to treat jaundice, rheumatoid arthritis and diabetes (Rajalakshmi *et al.*, 2009).

There are several individual reports on the antibacterial, antifungal and antioxidant activities of these three medicinal plants. However, there is no specific report about the phytochemical analysis, antimicrobial and antioxidant activities of individual plant parts such as the roots, stems and leaves of these three plants, which are important because all the plant parts were traditionally used in various concepts and treatments of several diseases. Therefore, we investigated the phytochemical constituents and the antibacterial, antifungal and antioxidant activities of various extracts of the roots, stems and leaves of these three important medicinal plants.

MATERIALS AND METHODS

Collection of plant materials: The roots, stems and leaves of *H. indicus*, *T. cordifolia* and *O. sanctum* plants were collected from the forest in the Kadiri region of the Chittoor district of Andhra Pradesh, India. The plant specimens were verified by Dr. Madhava Setty, a botanist from the Department of Botany, S. V. University, Tirupati, India.

Preparation of the extracts: All parts (roots, stems and leaves) of the three herbs were shade-dried and milled into fine powder using a mechanical grinder (TTK Prestige, Chennai, India). The ground plant material (100 g) was macerated and shaken in different solvents such as chloroform (500 mL, for the chloroform extract) or methanol (500 mL, for the methanolic extract) using a bath shaker (Thermo Scientific, Mumbai, India) for 48 h. The extracts were filtered through filter study (Whatman No.1) and were dried under a vacuum and reduced pressure using a rotary evaporator at 40°C. The concentrate was then placed in aluminum foil before freeze drying. The residual extract was dissolved in sterile water (1 mL) before analysis.

Phytochemical analysis: Based on the method published by Harborne (1998), qualitative tests were conducted on the crude extracts obtained with either methanol or chloroform to determine the different phytochemical constituents present in the plant extracts. The tests were performed in triplicate for each plant type.

Test for alkaloids:

- **Dragendorff's test:** The extract (1 mL) was transferred into a test tube before adding a few drops of Dragendorff's reagent (potassium bismuth iodide). The formation of an orange precipitate indicated the presence of alkaloids
- **Wagner's test:** The extract (1 mL) was added to 2 mL of Wagner's reagent (iodine in potassium iodide). The presence of alkaloids was confirmed if a reddish-brown precipitate formed
- **Meyer's test:** The extract (1 mL) was added to 2 mL of Mayer's reagent (potassiummercuric iodide). The development of a pale whitish precipitate confirmed the presence of alkaloids.

Test for steroids:

- **Salkowski's test:** The plant extract (1 mL) was mixed with an equal volume of chloroform and was treated

with 2 mL of concentrated sulfuric acid. The formation of a red precipitate indicated the presence of steroids

- **Liebermann-Burchard's test:** The plant extract (1 mL) was dissolved in an equal volume of chloroform. To this mixture, 2 mL of concentrated sulfuric acid and 2 mL of acetic anhydride were added. The development of a green colored precipitate indicated the presence of steroids

Test for tannins:

- **Ferric chloride test:** The plant extract (1 mL) was mixed with an equal amount of ferric chloride. The presence of tannins was indicated by the development of a greenish black color
- **Gelatin test:** A few drops of a gelatin solution (1%) which was formed by immediate dissolving in warm water, were added to the plant extract (1 mL). The formation of a white precipitate confirmed the presence of tannins

Tests for flavonoids:

- **Shinoda tests:** A few fragments of magnesium metal were added to a test tube containing 2 mL of plant extract. Then, concentrated hydrochloric acid was added dropwise. A magenta color indicated the presence of flavonoids
- **Ferric chloride test:** A few drops of 10% ferric chloride solution was added to a test tube containing 2 mL of the plant extract. The development of a green-blue or violet coloration confirmed the presence of a phenolic hydroxyl group

Test for phenols:

- **Ferric chloride test:** Briefly, a few drops of neutral ferric chloride solution (5%, w/v in 90% alcohol) were added to the extract. A blackish green color indicated the presence of a phenolic group
- **Ellagic acid test:** The plant extracts were treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrite (NaNO₂) solution. The presence of phenols was confirmed when the solution developed a whitish yellow or muddy brown precipitate

Antibacterial activities: The antibacterial activities of the plant extracts against *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Proteus vulgaris* (*P. vulgaris*) were investigated by a disc

diffusion method (Rao *et al.*, 2010). Briefly, petri plates containing 15 mL of particular media were seeded with the selected microbial strains. The filter study discs were saturated individually with each extract (20 mg mL⁻¹) before being aseptically placed on the seeded agar medium (Hi-Media Pvt. Ltd., Mumbai). The medium was pre-swabbed with the test organisms and was incubated at 37°C for 24 h. The measurement of antimicrobial activity was based on the size of the zone of inhibition that formed around the discs. Three independent trials were conducted for each concentration. The zone of inhibition was measured (in mm) and the mean was calculated.

Antifungal activities: The antifungal activities of the plant extracts were determined against *Aspergillus niger* (*A. niger*) and *Aspergillus flavus* (*A. flavus*) by a disc diffusion method. The cultures were grown on Potato Dextrose Agar (PDA) for 48 h before inoculation on the PDA plates. The filter study discs were infused with various respective extracts (20 mg mL⁻¹) and were aseptically placed on the seeded potato dextrose agar medium (Hi-Media Pvt Ltd., Mumbai) that had been swabbed with the test organism. The plates were then incubated at 37°C for 48 h. The zone of inhibition was calculated (mm) by taking the mean result of triplicate readings.

Antioxidant activities: The antioxidant properties of each plant extract were studied by estimating the free radical-scavenging activity of the DPPH radical based on the method established by Ferreira *et al.* (2009). Briefly, each plant extract (1.0 mL) was mixed with a methanolic solution containing DPPH solution (2.7 mL, 0.024 mg mL⁻¹). The mixture was vigorously shaken and incubated for 60 min at room temperature in the dark until the absorbance of the solution remained unchanged. The level of reduction of DPPH radicals was determined by measuring the absorbance of the mixture at 517 nm. The Radical-Scavenging Activity (RSA) was calculated as the percentage of DPPH discoloration using the following equation:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where, A_c is absorbance of control and A_s is absorbance of the test sample.

Statistical analysis: The results were expressed as the Mean±SD for triplicate experiments. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey's test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Phytochemical analysis: All of the methanolic extracts of the roots, stem and leaves of *H. indicus*, *O. sanctum* and *T. cordifolia* contained alkaloids, flavonoids, phenolic compounds, steroids and tannins (Table 1). The chloroform extracts of the roots, stem and leaves of *O. sanctum* also contained all of the investigated constituents (alkaloids, flavonoids, phenolic compounds, steroids and tannins) while only the chloroform extract of *H. indicus* roots was found to contain all of the constituents. Therefore, our results indicate that methanol is a more suitable solvent for the extraction of all three plant types.

These various phytochemical constituents have many bioactive actions and could contribute to the antioxidant, antibacterial and antifungal activities of the plant parts investigated below.

Antibacterial and antifungal activities: The methanolic and chloroform extracts of *H. indicus*, *O. sanctum* and *T. cordifolia* had potential activities against gram-negative and gram-positive pathogenic bacteria and some fungi. Overall, the methanolic extracts showed stronger antimicrobial activities when compared to the chloroform extracts of the different plant parts.

The methanolic leaf extract of *O. sanctum* showed antibacterial activity against *B. subtilis*. This was followed by the methanolic root extracts of *H. indicus* and *T. cordifolia* (Fig. 1).

The methanolic extracts of the *H. indicus* root and leaf, the methanolic extract of the *O. sanctum* leaf and the methanolic extract of the *T. cordifolia* stem exhibited antibacterial activity against *S. aureus* (Fig. 2).

The methanolic extracts of the *H. indicus* root and leaf and the methanolic extract of the *O. sanctum* leaf showed activity against the gram negative bacteria *E. coli* (Fig. 3).

Table 1: Phytochemical analysis of the plant extracts

Plant metabolites	Tests	<i>Hemidesmus indicus</i>						<i>Tinospora cordifolia</i>						<i>Ocimum sanctum</i>					
		Root		Stem		Leaf		Root		Stem		Leaf		Root		Stem		Leaf	
		ME	CE	ME	CE	ME	CE	ME	CE	ME	CE	ME	CE	ME	CE	ME	CE	ME	CE
Alkaloids	Dragendroff's test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Wagner's test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Mayer's test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	Salkowski's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Liebermann-Burchard's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
	Gelatin test	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+
Flavonoids	Shinoda test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ferric chloride test	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	Ferric chloride test	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
	Ellagic acid test	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+

ME: Methanolic extract, CE: Chloroform extract, +: Present and -: Absent

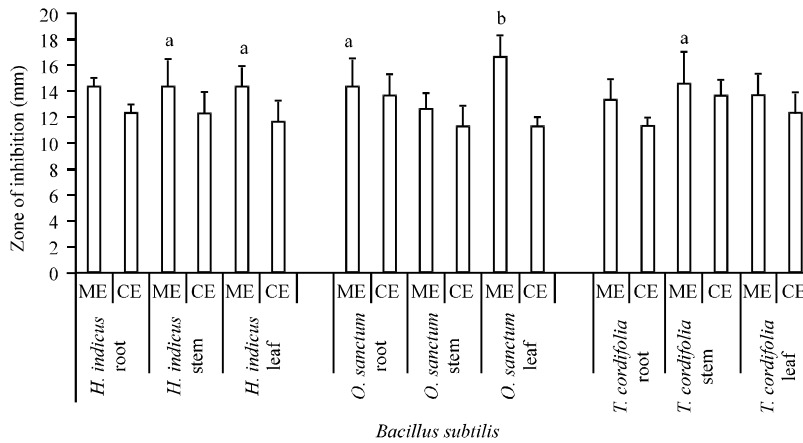


Fig. 1: Antibacterial activity of the plant extracts against *B. subtilis*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *B. subtilis*: *Bacillus subtilis*. Bars with the same superscripts do not differ significantly at $p < 0.05$

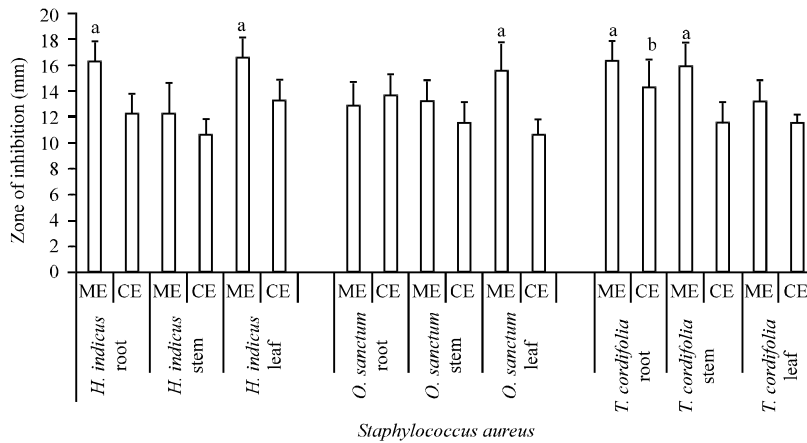


Fig. 2: Antibacterial activity of the plant extracts against *S. aureus*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *S. aureus*: *Staphylococcus aureus*. Bars with the same superscripts do not differ significantly at $p < 0.05$

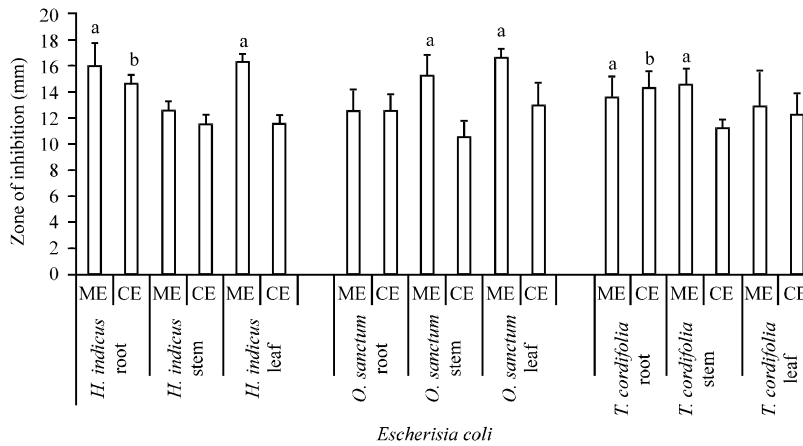


Fig. 3: Antibacterial activity of the plant extracts against *E. coli*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *S. aureus*: *Staphylococcus aureus*. Bars with the same superscripts do not differ significantly at $p < 0.05$

The chloroform extract of the *H. indicus* root showed the highest activity against *K. pneumoniae*, followed by the methanolic extracts of the *H. indicus* leaf and the *T. cordifolia* root (Fig. 4).

The methanolic extracts of the *H. indicus* and the *T. cordifolia* roots showed the highest activity against *P. vulgaris*, followed by the methanolic extract of the *H. indicus* stem (Fig. 5).

The methanolic extracts of the *T. cordifolia* root exhibited the highest activity against *A. niger*, followed by the methanolic extracts of the *H. indicus* root and stem and the *O. sanctum* stem (Fig. 6).

The *O. sanctum* root and leaf extracts showed the highest inhibition against *A. flavus* followed by the *T. cordifolia* root and leaf and the *H. indicus* stem methanolic extracts (Fig. 7).

Antioxidant activities: Overall, the methanolic plant extracts showed higher antioxidant activities than the chloroform extracts (Fig 8). The plants extracts that contained the highest antioxidant activity were the *H. indicus* methanolic root extract, the *O. sanctum* methanolic leaf extract and the *T. cordifolia* stem methanolic extract.

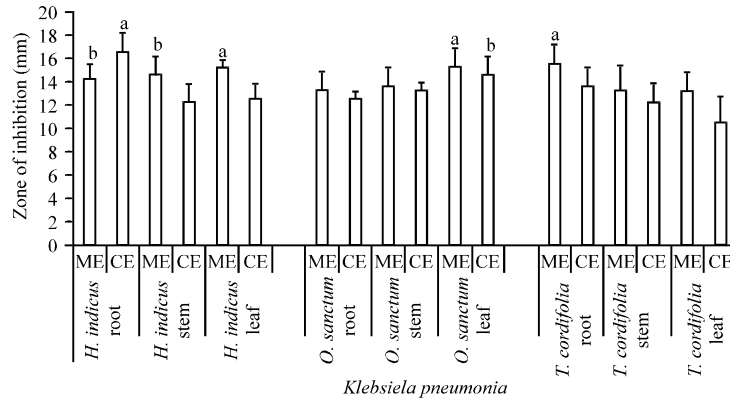


Fig. 4: Antibacterial activity of the plant extracts against *K. pneumoniae*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *K. pneumoniae*: *Klebsiella pneumoniae*. Bars with the same superscripts do not differ significantly at $p < 0.05$

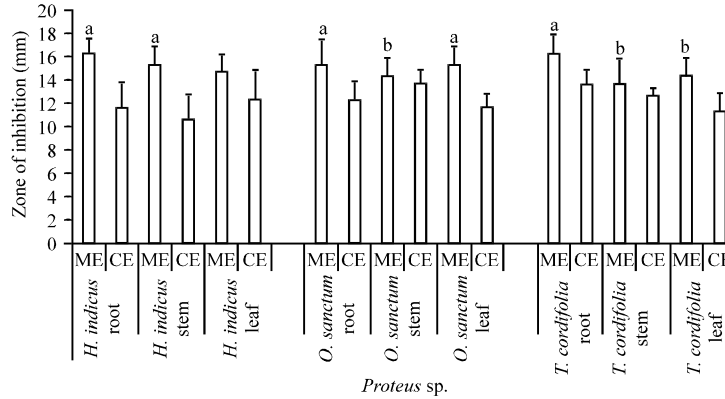


Fig. 5: Antibacterial activity of the plant extracts against *Proteus sp.* ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*. Bars with the same superscripts do not differ significantly at $p < 0.05$

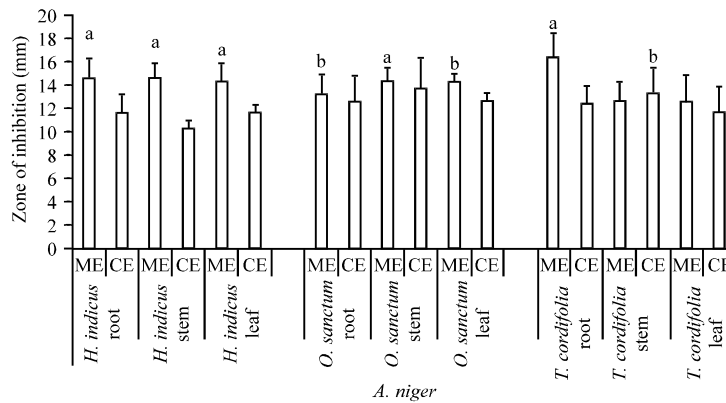


Fig. 6: Antifungal activity of the plant extracts against *A. niger*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *A. niger*: *Aspergillus niger*. Bars with the same superscripts do not differ significantly at $p < 0.05$

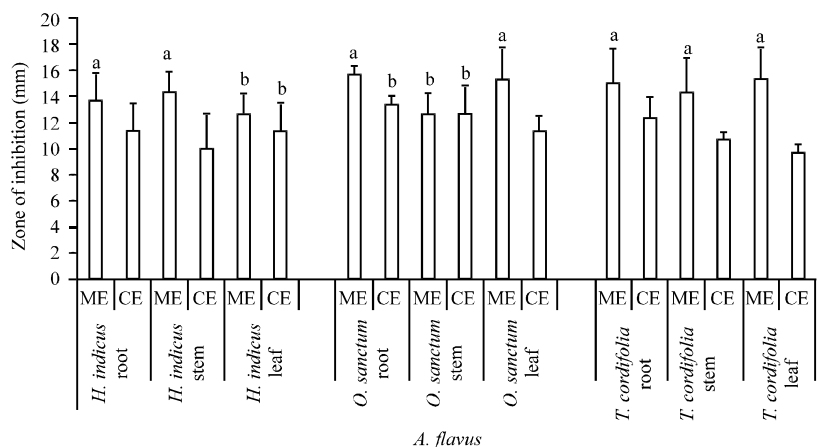


Fig. 7: Antibacterial activity of the plant extracts against *A. flavus*. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*, *A. flavus*: *Aspergillus flavus*. Bars with the same superscripts do not differ significantly at $p < 0.05$

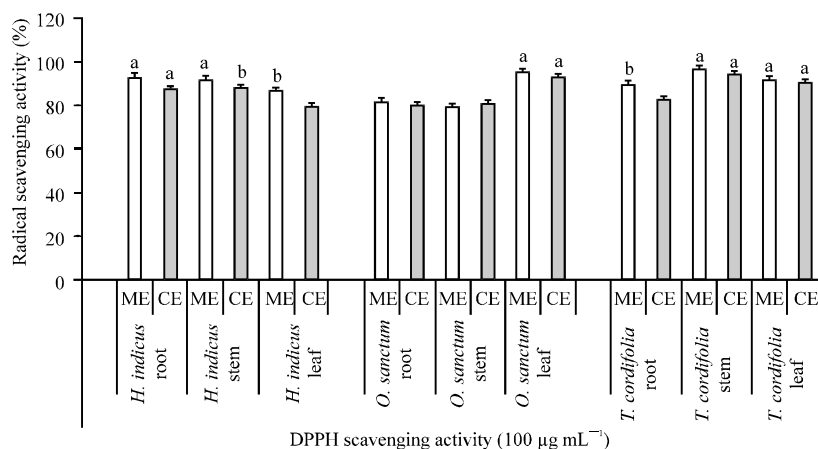


Fig. 8: Antioxidant activity of the plant extracts with DPPH assay. ME: Methanolic extract, CE: Chloroform extract, *H. indicus*: *Hemidesmus indicus*, *O. sanctum*: *Ocimum sanctum*, *T. cordifolia*: *Tinospora cordifolia*. Bars with the same superscripts do not differ significantly at $p < 0.05$

DISCUSSION

In this study, we report the phytochemical constituents present in *H. indicus*, *T. cordifolia* and *O. sanctum* stems, roots and leaves in two different extracts. The antimicrobial (antibacterial and antifungal) and the antioxidant activities of the different plants parts were also investigated. Overall, the methanolic extracts, which contained all of the investigated phytochemical constituents (alkaloids, steroids, tannins, flavonoids and phenols), exhibited higher antibacterial and antioxidant effects than the chloroform extract.

In a previous study, Kumar *et al.* (2007) reported that *H. indicus* contained tannins, flavonoids and phenols, which were also found in our study. However, Kumar *et al.* (2007) did not detect steroids or alkaloids, and this study used whole plants for analysis.

Tannins inhibit bacterial and fungal growth through non-specific binding to bacterial enzymes and direct action on pathogen metabolism by inhibiting oxidative phosphorylation or the capacity to complex transitional metals ions, which are essential for pathogen growth. Previous *in vitro* studies have indicated that tannins inhibit many strains of bacteria, including those from the genera *Aeromonas*, *Clostridium*, *Enterobacter*, *Bacillus*,

Escherichia, *Helicobacter*, *Proteus*, *Klebsiella*, *Shigella*, *Pseudomonas* and *Streptococcus*. Tannins can also inhibit fungi such as *Aspergillus* and *Penicillium* (Chung *et al.*, 1998).

Extensive side effect profiles and the development of microbial resistance to antimicrobial agents have elicited special attention towards the search of novel antimicrobial agents of natural origin. Several studies have established medicinal plants as an important source of active principles for drug formulation. Various reports have investigated the traditional use of medicinal plants against a number of infections (Gayathri and Kannabiran, 2009). The antibacterial activities of these plants are purported to be due to the various alkaloids in the plant extracts.

When comparing the various plant parts, the roots of *H. indicus* had the highest number of antimicrobial activities, showing antibacterial properties against all of the investigated organisms except for one (*A. flavus*). The methanolic and chloroform extracts of the *H. indicus* root contained many phytochemical constituents (alkaloids, steroids, tannins, flavonoids and phenols) and the DPPH test also confirmed that the *H. indicus* root has high antioxidant activity, which may contribute to the observed antibacterial activities.

Previous studies on the antibacterial activity of the *H. indicus* root aqueous extract showed potential activities against the Gram positive bacteria *S. aureus* and Gram negative bacteria such as *P. aeruginosa* and *K. pneumoniae*, while the ethanolic and chloroform extracts of *H. indicus* roots showed significant activities against *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *A. fumigates*, *A. flavus* and *A. niger* (Khanna and Kannabiran, 2008). The methanolic and chloroform extracts prepared from the roots of *H. indicus* are effective against enterobacterial growth (Das and Devaraj, 2006). Our results agree with the previous studies that show that the *H. indicus* root has significant activity against various pathogenic and opportunistic bacteria and fungi (Khanna and Kannabiran, 2008; Gayathri and Kannabiran, 2009).

Previous reports have confirmed that the roots of *H. indicus* have many biological activities when compared to other aerial parts of the plant. For example, the methanolic root extract of *H. indicus* was found to be hepatoprotective against paracetamol and chloroform-induced hepatic damage (Baheti *et al.*, 2006) and the ethanolic extract of the *H. indicus* root showed significant hepatoprotective activity against alcoholic liver damage.

The *H. indicus* leaf extract exhibited antibacterial activities against *S. aureus*, *E. coli* and *K. pneumoniae*, and the stem extract was effective against *P. vulgaris* and

A. niger. The stem of *H. indicus* contains many types of terpenoids, which play important roles in antimicrobial activities (Gupta *et al.*, 1992). Additionally, two novel pregnane glycosides, hemidescine and emidine, which have antioxidant and antimicrobial activities, have also been isolated from the *H. indicus* stem (Chandra *et al.*, 1994). In addition to the activity against the investigated micro-organisms, previous reports have indicated that methanolic extracts of *H. indicus* stems have antimutagenic activity against *S. typhimurium* (Aqil *et al.*, 2008).

The root of *T. cordifolia* has the second highest antimicrobial activity, acting against five different organisms (*B. subtilis*, *S. aureus*, *K. pneumoniae*, *P. vulgaris* and *A. niger*). In another study, the bark extracts of *T. cordifolia* were reported to have antimicrobial activities against *B. subtilis*, *E. coli*, *S. aureus*, *P. fluorescens* and *Xanthomonas axonopodis* and against some fungi such as *A. flavus*, *Dreschlera turcica* and *Fusarium verticillioides* (Mahesh and Satish, 2008). Previous reports have shown that the leaf extract of *T. cordifolia* exhibits antimicrobial properties against *B. subtilis*, *E. coli*, *Klebsiella aerogenes*, *P. vulgaris*, *Pseudomonas aeruginosa* and *S. aureus* (Samy and Ignacimuthu, 2000). Our results in the present study agree with the earlier reports that show the potential activities against these bacterial and fungal species (Samy and Ignacimuthu, 2000; Mahesh and Satish, 2008).

The root of *T. cordifolia* is also renowned for anti-stress activity. The aqueous extract of the root improves verbal learning and constant memory. The aqueous and alcoholic extracts of *T. cordifolia* both significantly improve the learning scores in the Hebb-Williams maze and memory retention, which indicates that this plant can improve learning and increase memory retention. Histopathological changes have indicated that *T. cordifolia* ameliorates neurodegeneration in the hippocampus of cyclosporin-treated rats (Agarwal *et al.*, 2002). The stem and root extracts of *T. cordifolia* are prescribed in combination with other drugs as an antidote to snake bites and scorpion stings. Oral administration of the *T. cordifolia* root aqueous extract reduces blood glucose levels and brain lipids significantly in alloxan diabetic rats. The *T. cordifolia* leaf extract also has potential hypoglycemic effects in normal and alloxan diabetic rabbits (Noreen *et al.*, 1992).

The *O. sanctum* leaf extract had antibacterial activity against *B. subtilis* and *E. coli*, but the stem extract is effective only against *B. subtilis*. In a previous report, the essential oil of *O. sanctum* exhibited activity against the four bacterial species *B. cereus*, *E. coli*, *Klebsiella* spp.

and *Pseudomonas* spp. (Aggarwal and Goyal, 2012). In another study, flavonoids exhibited activity against *E. coli*, *Proteus* spp., *S. aureus*, *Staphylococcus cohnii* and *Klebsiella pneumonia* (Ali and Dixit, 2012). suggested that the higher concentrations of linoleic acid in the fixed oil of *O. sanctum* could contribute to the antibacterial activity (Singh *et al.*, 2005). Our results for the *O. sanctum* leaf extracts agree with the previously reported studies (Parag *et al.*, 2010; Aggarwal and Goyal, 2012).

The antioxidant properties of numerous plant extracts have been investigated using whole sample and phenolic extracts of natural origin. Many tests have been developed to measure the antioxidant capacity of plant extracts, foods and biological samples. The antioxidant activity of plant extracts cannot be assessed by using a single method due to the complex nature of phytochemicals. Therefore, it is essential to use commonly accepted assays to measure the antioxidant activity of the plant extract. DPPH scavenging activity is one of the most universally accepted methods for measuring antioxidant activity. DPPH is a stable free radical that gives deep violet color in methanol solution illustrated by an absorption band centered at 517 nm. The principle behind the DPPH assay is that the antioxidants donate hydrogen and reduce the stable DPPH radical to a yellow-colored, non-radical form of para-hydroxy-derivatives of 2,2-diphenyl-1-picrylhydrazine (DPPH-H) (Yang *et al.*, 2009).

Numerous natural compounds have been recognized with widespread biological activities, with special attention on polyphenols, which have extremely important potential applications in medicine. Polyphenols are a major class of substances with more than 8000 compounds, including simple structures and polymeric compounds such as tannins. Flavonoids are also an extremely vital and known group of compounds with a range of pharmacological effects. Flavonoids affect membrane permeability and inhibit membrane-bound enzymes such as ATPase and phospholipase A2 (Aiyegoro and Okoh, 2010). These observations support the effectiveness of medicinal plants in traditional remedies for the treatment of stress-related ailments as antioxidants.

In recent years, many studies have been carried out on the beneficial effects of phenolic compounds as natural antioxidants that counteract the action of free radicals. Phenolic acids act as antioxidants through free radical trapping action. The antioxidant mechanism of phenolic compounds is due to their ability to chelate metal ions. Phenolic compounds possess carboxyl and hydroxyl

groups that are able to bind copper and iron (Jung *et al.*, 2003). Phenolic compounds have diverse antioxidant abilities that are dependent upon the structural activity, number of hydroxyl groups and the allocation of these groups in the structure. Plant roots that are exposed to heavy metals often exhibit high levels of phenolics. These compounds lessen the capacity of iron ions by a chelating mechanism and diminish the superoxide-driven Fenton reaction, a large source of ROS. Flavonoids can reduce lipid oxidation through free radical scavenging and metal chelating mechanisms (Gheldof *et al.*, 2002). In addition, flavonoids also play an important role as pro-oxidants in the presence of transition metal ions. Flavonoids constantly diminish these ions, and this process generates OH• via., the Fenton reaction.

Our study has confirmed that the *O. sanctum* leaf extract has antioxidant potential as indicated by the DPPH test. Another study investigating the *O. sanctum* leaf hydroalcoholic extract produced similar results (Kath and Gupta, 2006). Previous reports conducted on the ethanolic and aqueous extracts of *O. sanctum* also showed potential antioxidant activity, thyroid function regulation and cyclooxygenase inhibitory activity. The seed oil of *O. sanctum* also has been reported to have antidiabetic, antioxidant, anticholesteremic activities (Kath and Gupta, 2006).

In this study, we also found that the methanolic root and stem extracts of *H. indicus* have antioxidant potential as indicated by the DPPH radical scavenging activity assay. In a previous study, the aqueous ethanolic root extracts of *H. indicus* considerably abridged the formation of gastric and duodenal damage induced by a variety of ulcerogenic and cytotoxic agents in rats (Anoop and Jegadeesan, 2003). The antiulcer activity of this plant extract may be due to the presence of terpenoids, saponins and amino acids. Many studies have documented the antioxidant activities of *H. indicus*. The root extracts of *H. indicus* protect against radiation and DNA damage (Shetty *et al.*, 2005). In addition to *H. indicus*, the stem extract of *T. cordifolia* also has antioxidant potential. The DPPH activity indicated that these plants may contain compounds that donate hydrogen to a free radical to eliminate the odd electrons that are responsible for radical reactivity. The ability of these plant extracts to forage DPPH could indicate that these plant extracts could be used to treat radical-related pathological disturbances, especially at higher concentrations.

Due to the antimicrobial and antioxidant potential, these herbs, especially the roots of *H. indicus* and *T. cordifolia*, should be further studied as new sources of inexpensive and effective anti-microbial and antioxidant

agents. In this regard, bioassay-oriented fractionation of the active compounds should be conducted not only on the methanolic extracts but also on the chloroform extracts of the various parts. Additionally, other solvents such as water and ethanol should also be investigated.

CONCLUSION

The methanolic extracts of all three plants exhibited both higher antibacterial and antioxidant effects when compared to the chloroform extracts. This may be related to the high content of phytochemical constituents such as alkaloids, steroids, tannins, flavonoids and phenols. When compared the various plant parts, the roots of *H. indicus* had the highest number of antimicrobial activities, showing antibacterial properties against all of the investigated organisms except one (*A. flavus*). The *H. indicus* leaf extract exhibited antibacterial activities against *S. aureus*, *E. coli* and *K. pneumoniae* while the stem extract was effective against *P. vulgaris* and *A. niger*. The root of *T. cordifolia* had the second highest antimicrobial activity, acting against five different organisms (*B. subtilis*, *S. aureus*, *K. pneumoniae*, *P. vulgaris* and *A. niger*). The *O. sanctum* leaf extract showed antibacterial activities against *B. subtilis* and *E. coli*, while the stem extracts were only effective against *B. subtilis*. The methanolic *H. indicus* root and stem extracts, the methanolic *O. sanctum* leaf extract and the methanolic *T. cordifolia* stem extract also exhibited antioxidant potential. All the three plant extracts exhibited potential antimicrobial and antioxidant activities in all the tested solvents and thus, these plants may be of great interest to the development of new drugs.

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