



ANTIMICROBIAL ACTIVITY OF *TINOSPORA CRISPA* ROOT EXTRACTS

Asif Iqbal Chittur Mohammed^{1*}, Gunjan Manish¹, Chellappan Kumar Dinesh²

¹Faculty of Pharmacy, Masterskill University College of Health Sciences, Malaysia

²Department of Life Sciences, International Medical University, Malaysia

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*Corresponding author

Email: asiffeb19@gmail.com

ABSTRACT

The aim of this study was to determine the *in vitro* antimicrobial activity of ethanol, distilled water, methanol and chloroform crude extracts of the roots of *Tinospora crispa*. Antimicrobial activity was examined by disc diffusion method against gram positive bacterial strains of *Streptococcus pneumonia*, gram negative bacterial strains of *Escherichia coli* and fungal strains of *Candida albicans*. The maximum zone of inhibition was obtained with ethanol extract against *Escherichia coli* and *Streptococcus pneumonia* followed by chloroform extract against the same organisms. Whilst distilled water extract showed a minimal zone of inhibition, methanol extract showed a moderate zone of inhibition against the bacterial strains used. The values were compared with a standard antibiotic. The ethanol extract also showed the maximum zone of inhibition against the growth of *Candida albicans*, whereas the lowest activity was shown with distilled water crude extract. Methanol and chloroform crude extracts showed considerably moderate activities against the fungal strain, as compared to the standard antibiotic used.

Keywords: *Tinospora crispa* root, crude extracts, antimicrobial activity, disc diffusion method

INTRODUCTION

Plants and plant products have long been used as principal ingredients that aid human health and well-being. The plant *Tinospora crispa* (Family-Menispermaceae) occupies a very important place in the field of medicinal plants and is widely used as a traditional medicine. *Tinospora crispa* (*T. crispa*) is a small herb which grows widely in temperate and tropical parts of Asia. More specifically, the plant is widely found in tropical and subtropical Philippines, Indonesia, Malaysia, Thailand, India, China and Vietnam. The plant is also known by its numerous synonyms, *viz.*, *Menispermum crispum* Linn., *Tinospora cordifolia* F. Vill., *Tinospora tuberculata*, *Tinospora rumphii* and other local names like Makabuhai, Andawali, Putarwali, Kattukkodi, Vasanavalli, Boraphet and Wan kab hoi yai. *T. crispa* is attributed to its ethno-medical uses since ancient times by traditional healers and physicians. Local people use this plant for the treatment of skin diseases. It is also used for many other cures; as an antiperiodic in fever, as a tonic, alternative, diuretic, oral hypoglycemic agent, wound cleansing agent for chronic rheumatic wounds, in malarial fever and in intestinal worm infestation. Chemically, *T. crispa* contains a bitter principle, columbine, which is glucosidal in nature. It also contains picoretine, berberine, an alkaloid, triterpenes namely cycloeucaenol, cycloeucaenone and flavones-O-glycosides like apigenin and resin¹. Apart from the presence of different chemical components, the plant *T. crispa* also possess a wide spectrum of pharmacological properties, which are reported in the literature *viz.*, antibacterial², antinociceptive, anti-inflammatory³, antiproliferative⁴ and antioxidant⁵. As of date, there are no available literatures on the antibacterial potential of the root extracts of *T. crispa*.

Although there are many antimicrobial medicinal plants and plant products which are used widely today, the search is still on for a comprehensive, potent, broad spectrum antimicrobial drug of natural origin. Based on the traditional uses and in view of the above observation,

we thought it was worthwhile to carry out a study on the *in vitro* antimicrobial activity of aqueous, ethanol, methanol and chloroform extracts of the roots of *T. crispa* against selected gram positive and negative bacterial and fungal strains.

MATERIALS AND METHODS

T. crispa roots were collected around Tali Air 8, Sungai Leman Sekinchan area in Selangor, Malaysia (voucher specimen no MUCH/HPH/T2/003). Collected roots were washed under running water, to remove any clay and mud, then cut into small pieces³ and oven-dried for approximately 7 days at 40°C, until there were no changes in weight after three successive readings taken at regular intervals.

Dried cut pieces of roots were then grounded into coarse powder form by using a blender and stored in a well closed container until further used. Extraction was carried out individually for each solvent by cold maceration of the root powder of *T. crispa* to obtain the respective crude extracts of distilled water (DH₂OExt), methanol (MeOHExt), ethanol (EtOHExt) and chloroform (ChExt). Maceration was done for each solvent in the ratio of 1:20 (w/v) for 24 hours at room temperature. The soaking waste residues were filtered off by using a Whatman filter paper (No. 42) to obtain the respective crude extract filtrates.

The collected extract filtrates were stored in a refrigerator (-20°C) for subsequent use. The refrigerated extract filtrates were then evaporated in a rotavapor to dryness under reduced pressure and the crude dried extracts were weighed and then suspended in 20 mL of carboxy methyl cellulose (CMC) solution, which were considered as stock solutions (100% concentration). The resultant distilled water extract was directly considered as a stock solution of 100% concentration.

All stock solutions of the respective crude extracts were suspended in distilled water, in the concentration of 50µg/mL. 20 µl each of EtOHExt, MeOHExt, ChExt and

DH₂O extracts were then weighed and transferred into blank sterilized discs (Grade AA discs with 6mm diameter, Whatman International Ltd., England). The discs were then subjected to antimicrobial screening using disc diffusion method against standard antibiotic discs (tetracycline 30µg/disc and fluconazole 25µg/disc) which were used for comparison².

The microorganisms used in the study were *Streptococcus pneumonia* (*S. pneumonia*), *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*). Nutrient broth Mueller Hinton Agar (MHA) (HiMedia Laboratories Pvt. Ltd. India) was used for *S. pneumonia* and *E. coli* and Sabouraud's agar (HiMedia Laboratories Pvt. Ltd. India) for *C. albicans*. The micro organisms were incubated at 37°C±0.5 for 24 hours after injection into their respective media.

The media were sterilized prior to use by autoclaving (ES-215 Tomy Seiko Co Ltd, Tokyo, Japan) at 15 lbs pressure (121°C) for 15 minutes and were poured (15mL) into sterilized petri dishes (diameter of 9cm) after which they were allowed to cool at room temperature and solidify.

This was followed by swabbing the bacterial culture onto medium in petri dishes. Discs impregnated with extracts were then placed on the solid agar medium by pressing evenly. Petri dishes were placed in an incubator (Model:

1B-01E/11E/21E Jeio Tech, Korea) at 37°C±0.5 for 24 hours for the bacterial strains and at 27°C for 48 hours for the fungi *C. albicans*, according to the specified requirements of temperature and conditions for growth of respective organisms. After the specified time, inhibition zones formed were measured in mm. The inhibition zones were compared with the inhibition zones observed for standard antibiotic discs.

RESULTS AND DISCUSSION

In this work, the antimicrobial effects of *T. crisper* crude extracts of root were studied using disc diffusion method. The results showed different sensitivity levels for the tested strains of *E. coli*, *S. pneumonia* and *C. albicans*, and the inhibition zones ranged between 0.9 ± 0.0 to 3.8 ± 0.2 mm.

Among the three tested strains, EtOHExt and ChExt were found to have better antimicrobial effect compared to that of DH₂OExt and MeOHExt. Standard tetracycline showed 13 to 19mm zone of inhibition for *E. coli*, *S. pneumonia* and standard fluconazole showed a 6mm zone of inhibition for *C. albicans* (Table 1). However, it is important to bear in mind that all the extracts used were in crude forms. We believe that purified forms of isolated compounds from these extracts would show more potential activities.

Table 1: Antimicrobial activity of crude extracts of *Tinospora crispa* root by disc diffusion method

	Zone of Inhibition (mm)		
	<i>E. coli</i>	<i>S. pneumonia</i>	<i>C. albicans</i>
DH ₂ OExt	2.6±0.5	2.8±0.2	1.8±0.2
EtOHExt	3.6±0.2	3.5±0.5	3.8±0.2
MeOHExt	0.9±0.0	1.9±0.1	2.0±0.3
ChExt	2.8±0.2	3.5±0.5	2.3±0.2
Tetracycline	13±0.0	19±0.0	NA
Fluconazole	NA	NA	6±0.0

All values are Mean ± Standard Deviation. N=3 (the experiment was performed in triplicate). DH₂OExt=Distilled water extract, EtOHExt=Ethanol extract, MeOHExt=Methanolic extract, ChExt=Chloroform extract. NA= Not Applicable.

It is generally known that crude extracts might contain various types of active compounds, such as tannins, flavonoids⁶, glycosides⁷ or saponins⁸ to name a few. The presence of the above mentioned compounds abundantly in all types of plants⁸ might help explain the observed antimicrobial activity of *T. crisper* root extracts.

Zakaria *et al.*, 2006 conducted a study on the in-vitro antibacterial potential of *T. crisper* stem crude extracts and found moderate antibacterial activity. It may be inferred that the same constituents may be responsible for the antimicrobial activity of the root extracts as well. The active antimicrobial compounds present in the stem extracts may also be present in the roots.

T. crisper contains many flavones and flavonoids and it is well demonstrated that flavonoids are potent antioxidants⁵; hence it can also be corroborated that the antimicrobial activity found in *T. crisper* root extracts may also be due to the presence of flavonoids. The stem extracts of *T. crisper* has already been proven for its antioxidants effects⁵.

Phytoconstituents present in plants namely flavonoids, alkaloids, tannins and triterpenoids are producing exciting opportunities for the expansion of modern chemotherapies against wide range of microorganisms^{9,10} and our study

has warranted these claims based on the previously determined chemical constituents of *T. crisper*. It is plausible to suggest that the observed antimicrobial activity could be attributed to the presence and synergistic action of flavonoids and alkaloids.

CONCLUSION

In conclusion, this study showed the basic information on the antimicrobial potential of *T. crisper* root extracts with variable responses. Further studies are required to substantiate new biologically potent active antimicrobial compounds from *T. crisper* root.

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