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Acute toxicity and antifungal studies of ethanolic leaves, stem and pulp extract of *Tamarindus indica*

[1]Abubakar MG, [2]Yerima MB, [2]Zahriya AG and [1, 3]Ukwuani AN*

[1]Department of Biochemistry, Faculty of science, Usmanu Danfodiyo University, Sokoto
[2]Department of Microbiology, Faculty of science, Usmanu Danfodiyo University, Sokoto
[3]Department of Biochemistry, Faculty of science, Kebbi State University of Science and Technology, Aliero.

ABSTRACT

The acute oral toxicity studies of the pulp extract of *Tamarindus indica* at 3000mg/kg and 5000mg/kg body weight of resulted in no mortality. This suggests that the LD_{50} is greater than 5000mg/kg body weight and can be classified as practically non-toxic and considered safe by the recommendations of World Health Organization (WHO) and Organization for Economic and Cultural Development (OECD). Antifungal activity of ethanolic extract of *Tamarindus indica* (leaves, stem bark and pulp) against *A. niger*, *A.flavus* and *F.oxysporum* was studied. The result showed a dose dependent increase in inhibition of growth of these organisms. Of the three(3) plant part the stem bark did not inhibit growth of *A. niger* and slightly inhibited the growth of *A.flavus* and *F.oxysporum* . From this study we can conclude that the pulp and especially the leaves of *T.indica* could be a promising antifungal agent and the result confirms the use of this plant in traditional medicine for the treatment of fungal infections.

Key word: *Tamarindus indica,* $LD_{50,}$ acute oral toxicity studies, pulp.

Corresponding author

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INTRODUCTION

The recorded use of plants in the treatment of ailments dates back to antiquity (Sofowora, 1993). Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Cragg et al. 1999). Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines (Verpoorte 1998, 2000).

Herbal medicines already form the basis of therapeutic use in developing countries but recent years have also seen an increase in the use of herbal medications in the developed world as well. Some studies focusing on the investigation of traditional African (Kudi *et al*., 1999; Okeke *et al*., 2001), Caribbean (Chariandy *et al*., 1999) and Indian (Ahmad and Beg, 2001) medicinal plants have resulted in the identification of new sources of therapeutic agents. Antimicrobial multiple drug resistance toward commonly used commercial drugs has resulted in an increase in the search for antimicrobial agents from natural sources. Plant derived antimicrobial agents are a largely untapped resource with enormous medical potential and much more investigation is needed in this area.

Infective diseases account for approximately one-half of all death in tropics (Iwu et al., 1999). In the area of antiinfectives about 70% are naturally derived (Cragg and Newman, 2005). The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic chemotypes. Nigeria's diverse flora offers a wide spectrum of medicinal plants. Tamarind (*Tamarindus indica*, Fabaceae), a tropical fruit found in Africa and Asia is highly valued for its pulp. Tamarind fruit pulp has a sweet acidic taste due to a combination of high contents of tartaric acid and reducing sugars (Morton, 1987). The pulp is used for seasoning, in prepared foods, to flavour confections, curries and sauces, and as a major ingredient in juices and other beverages (Abubakar *et al*, 2008a). Leaves and flowers can be eaten as vegetables, and are prepared in a variety of dishes. They are used to make curries, salads, stews and soups.

Tamarind is also extensively used in Nigerian traditional medicine especially in the north-western region. It has been reported to be among the recipe in the treatment of cold, fevers, stomach disorders, diarrhoea, jaundice and as skin cleanser (Doughari, 2006). It is applied on inflammations, used to gargle sour throat, mixed with salt as a liniment for rheumatism, relieve pains, reduce secondary bacterial infection and promote healing (Fabiyi et al, 1993). Our previous research has shown the pulp extract to be antibacterial (Abubakar *et al*, 2008a), cholesterol and Low density lipoprotein (LDL) reducing agent (Ukwuani *et al*, 2008) and above all relatively safe for human consumption ((Abubakar *et al*, 2008b)

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The present study was therefore carried out to evaluate the safety and antifungal properties of ethanolic extracts of *Tamarindus indica* pulp, leaves and stem-bark.

MATERIALS AND METHODS

Plant Material

Tamarindus indica pulp was obtained from Sokoto state central market while the leaves and stem bark were obtained from the wild of Sokoto south local government area of Sokoto state, Nigeria. These plant parts were identified at Botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria. A voucher specimen was prepared and deposited in the herbarium of the same institution for reference as recommended by Kumar *et al*. (2000).

Preparation of Extract

The pulp, leaves and stem bark ethanolic extracts of *Tamarindus indica* were obtained using the activity guided fractionation method as described by Brian and Tinners (1999). One hundred gram (100g) each of grounded fine powder of the plant parts was soaked in 500mL of ethanol. This was shaken for 10 min and allowed to stand for 24 hours then filtered. The filtrate was evaporated in a rotary evaporator and dried in to a residue in a drying cabinet at 40^0C.

Acute oral toxicity studies

A total of 10 adult Wister *albino* rats weighing between 202 ± 55g were obtained from the colony bred at the zoological garden of Usmanu Danfodiyo University, Sokoto. They were house in metal cages at the Research Laboratory, Pharmacology Department of the same institution. They were fed on pellets of growers mash poultry feed produced by Vital Feeds (Jos), and allowed free access to tap water for a period of two weeks for acclimatization. After acclimatization period, two groups of 5 rats each were dosed individually at 48 hours interval with 3000mg/kg and 5000mg/kg body weight *T. indica* pulp extract respectively using the up and down procedure as described by Dixon (1991). The behavioural changes and other changes observed in animals were recorded according to Organization for Economic and Cultural Development (OECD) 425 guidelines as described by Dixon (1991). Subsequently all animals were observed for the next 14 days for any delayed toxic effects.

Media preparation

Sixty five grams (65g) of sabouraud dextrose agar (SDA) was suspended in one litre of distilled water and swirled continuously for even distribution. This was then sterilised in an autoclave at 120^oC for 15 minutes and allowed to cool.

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Test organism

Clinical isolates were obtained from the Mycology Unit, Microbiology Department, Usmanu Danfodiyo University Teaching Hospital (UDUTH) and reidentified according to the method of Cowas and Steel (1992).

Antifungal sensitivity test

This was carried out according to the method described by Cheesebourg (2000). Fifteen (15ml) of agar and 5ml of sample at varying concentrations were poured in a sterile conical flask and swirled for proper mixing after which it was transferred in to petridishes aseptically to solidify. The isolates were then inoculated on each plate and incubated at room temperature for 14 days.

RESULTS

The results of the acute oral toxicity study of the pulp resulted in no mortality and other behavioural changes observed are recorded in table 1 below. The effect of *T. indica* leaves, pulp and stem-bark extracts on *A. Niger* is shown in Table 2. All plant part except stem-bark showed a dose dependent antifungal effect when compared to the positive control (fulcin). In Table 3, the result showed that *A. Flavus* was more susceptibly to the leaves and pulp than the stem-bark which only inhibited the growth at the lowest dose (5mg/ml). F.oxysporum on the other hand showed to be the most susceptible to all the plant part (Table 4). The zones of inhibition were dose dependent but in the pulp and stem-bark, antifungal effect was recorded at doses of 20mg/ml and above only.

Table1: Behavioural changes observed in the acute oral toxicity studies

GROUP	DOSE	NO. OF DEATH	BEHAVOIURAL CHANGES
A	3000mg/kg	0	Mild restlessness, erection of hair coat, Increased respiratory rate in the first 5 minutes, confusion, scratching of their nostrils and head, anorexia, Sensitive to slight sound, moving around restlessly and whipping of their mouths.
B	5000ng/kg	0	Moderate behavioral changes as seen in A.

Table 2: Result of the percentage inhibition in the mycelia growth of *A. Niger*

Plant part	% inhibition (mm) at varying concentration			
	5(mg/ml)	20(mg/ml)	40(mg/ml)	80(mg/ml)

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Leaves	39	40	61	76
Pulp	27	46	64	73
Stem bark	0	0	0	0
Fulcin	92	98	98	98
SDA	0	0	0	0

Table 3: Result of the percentage inhibition in the mycelia growth of *A. flavus*

Plant part	**% inhibition (mm) at varying concentration**			
	5(mg/ml)	**20(mg/ml)**	**40(mg/ml)**	**80(mg/ml)**
Leaves	36	40	61	76
Pulp	49	46	64	73
Stem bark	31	0	0	0
Fulcin	90	92	98	98
SDA	0	0	0	0

Table 4: Result of the percentage inhibition in the mycelia growth of *F.oxysporum*

Plant part	**% inhibition (mm) at varying concentration**			
	5(mg/ml)	**20(mg/ml)**	**40(mg/ml)**	**80(mg/ml)**
Leaves	39	46	72	82
Pulp	0	34	48	75
Stem bark	0	27	66	80
Fulcin	86	90	98	98
SDA	0	0	0	0

DISCUSSION

The oral acute administration of 3000mg/kg and 5000mg/kg body weight of the pulp extract of *Tamarindus indica* resulted in no mortality. This suggests that the LD_{50} is greater than 5000mg/kg and can be classified as practically non-toxic using the Hamburger' s (1989) classification of range of LD_{50}. However, behavioral changes observed in this study may be attributed to secondary metabolite content of the extract such as saponin (Abubakar et al (2008a) since its toxicity effects includes; fatigue, malaise, confusion and anorexia(Goodman and Gilman, 1996). LD_{50} greater than 5g/kg body weight is considered safe by the recommendations of World Health Organization (WHO) and Organization for Economic and Cultural Development (OECD) guideline respectively.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al*., 1998) which we have earlier reported (Abubakar *et al*, 2008). Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants (Mahesh and Satish, 2008). Some of these observations have helped in identifying the active principle responsible for such activities and in the development of drugs for therapeutic use in human beings.

Fungal related diseases may not be as common as other microbial infections but, when present, they are difficult to treat especially in immunosuppressed persons (Bryce, 1992). The treatment given by the traditional doctors often includes the administration of entire plants, or extracts of roots, stems, bark, leaves, fruits, seeds or juice of the plant. The treatment might be wrong sometimes, hence the need to scientifically analyze the medicinal plants for their efficacy. Antifungal activity of several Nigerian plants has been investigated and documented. These include that of *Mitracarpus villosus* (Irobi and Daramola, 1993), *Ritchiea capparoides* (Ajaiyeoba *et al.*., 1998) and *Khaya ivorensis* and *Tetracera potatoria* (Adekunle *et al*., 2003).

This study revealed a dose dependent inhibition of growth of the test organisms with the leaves showing the overall highest activity in all test organisms while the stem bark showed activity only on *F.oxysporum*. The success of the ethnobotanical approach to drugs discovery can no longer be questioned. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but may be due to the combine action of different compounds originally in the plant (Bai, 1990). Since plants contain secondary metabolite that could induce toxic effects to invading organisms, there is the need for phytochemical analysis and standardisation of this plant through acute, sub-acute and chronic toxicity testing with a view to ascertain its safety. Further screening should be conducted on other test organism.

The results of present investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of plants used in the Hausa tradition medicine in

Nigeria for the treatment of fungal infection, which could be of considerable interest to the development of new drugs.

REFERENCES

[1] Abubakar, M.G., Ukwuani A. N. and Shehu R. A. (2008). An evaluation of the toxic effect of Tamarindus indica pulp in rats. Journal of pharmacology and toxicology. 3(2): 111-118.

[2] Abubakar, M.G., Ukwuani A. N. and Shehu R. A. (2008). Phytochemical and antibacterial screening of pulp of Tamarindus indica in rats. Asian Journal of Biochemistry. 3(2): 134-138.

[3] Ukwuani, A. N., Abubakar, M. G., Shehu, R. A. and Hassan, L. G. (2008). Antiobesity effects of pulp extract of Tamarindus indica pulp in rats. Journal of pharmacology and toxicology. 3(4): 221-227.

[4] Fabiyi, J. P., Kela, S. L., Tal, K. M., and Istifamus, W. A. (1993). Traditional therapy of dracunueliasis in the state of bauchi, Nigeria. Dakar Med., 38: 193 – 195.

[5] Sofowora, A. E. (1993). Medicinal Plants and traditional medicine in Africa. Vol 2. Spectrum Books Ltd, Ibadan; pp 288.

[6] Doughari, J. H. (2006). Animicrobial activity of tamarindus indica linn. Trop. J. Pharm. Res., 5: 597

[7] Morton, J., (1987). Tamarinds. In: fruits of the cimates. Pp 115 – 121. Maimi, FL

[8] Adekunle A. A. (2001). Ethnobotanical studies of some medicinal plants from Lagos State of Nigeria. Nigerian Journal of Botany. 14: 71 – 79.

[9] Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World Journal of Agricultural Sciences 4 (S): 839-843.

[10] Tona, L., Kambu, K., Ngimbi, N., Cimanga, K. and Vlietinck, Z. A. (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol., 61: 57-65.

[11] Burkill, H. M. (1997). The useful plants of the west tropical Africa. Vol 2. Royal Botanic Garden, Kew. Pp: 969.

[12] Oliver, B. E. P.(1960). Medicinal Plants in Nigeria. Nigerian College of Arts, Science and Technology, Lagos; pp: 70.

[13] Keay, R. W. J., Onochie, C. F. A. and Stanfield, D. F. (1964). Nigerian Trees. Nigeria National Press Ltd, Lagos; pp: 495.

[14] Sofowora, A. E. (1982). Medicinal plants and traditional medicine in Africa Vol 1. John Wiley and Sons, New York; pp: 251.

[15] Bryce, K. (1992). The Fifth kingdom. Mycologue Publications, Ontario; pp: 451

[16] Irobi, O. N. and Daramola, S. O. (1993). Antifungal activities of crude extracts of Mitracarpus villosus (Rubiaceae). J Ethnopharmacol. 40**:** 137–140.

[17] Ajaiyeoba, E. O., Rahman, A. W. and Chondhary, I. M. (1998). Preliminary antifungal and cytotoxicity studies of extracts of Ritchiea capparoides var. longipedicallata. J Ethnophamacol. 62**:** 243–246.

[18] Adekunle, A. A., Duru, C. and Odufuwa, O. M. (2003). Antifungal activity and phytochemcial screening of the crude extracts of Khaya ivorensis JUSS (Meliaceae) and Tetracera potatoria L (Dilleniaceae). South African Journal of Botany. 69**:** 568–571.

[19] Iwu, M. W., Duncan, A. R. and Okunji, C. O. (1999). New Antimicrobials of Plant origin In: J.Janick (ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA. pp. 457-462.

[20] Cragg, G.M. and Newman, D. J. (2005). Biodiversity: A continuing source of novel drug leads. Pure Appl. Chem., 77(1): 7–24.

[21] Cragg, G. M., Boyd, M. R., Khanna, R., Kneller, R., Mays, T. D., Mazan, K. D., Newman, D. J. and Sausville, E. A. (1999). International collaboration in drug discovery and development: the NCI experience. Pure Appl Chem 71: 1619-1633.

[22] Verpoorte, R. (1998). Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Develop Trends 3: 232-238.

[23] Verpoorte, R. (2000). Pharmacognosy in the new millennium: lead finding and biotechnology. J Pharm Pharmacol 52: 253-262.

[24] Chiariandy, C. M., Seaforth, C. E., Phelps, R. H., Pollard, G. V. and Khambay, B. P. (1999). Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of Ethnopharmacology, 64, 265-270.

[25] Ahmad, I. and Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens Journal of Ethnopharmacology, 74, 113-123.

[26] Kudi, A. C., Uhoh, J. U., Eduvie, L. O. and Gefu, J. (1999). Screening of some Nigerian medicinal plants for antibacterial activity. Journal of Ethnopharmacology, 67, 225-228.

[27] Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli, A. S. and Esimone, C. O. (2001). Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. Journal of Ethnopharmacology, 78, 119-127.

[28] Cowas, S. T. and Steel, J. R. (1992). Mannual for the identification of medical bacteria. University press. Cambridge 3rd edition.