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

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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<sub>50</sub> 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<sub>50</sub>, acute oral toxicity studies, pulp.

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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.

Infecrive 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°C.

**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°C for 15 minutes and allowed to cool.
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 Cheesebourough (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.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DOSE (mg/kg)</th>
<th>NO. OF DEATH</th>
<th>BEHAVIOURAL CHANGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3000</td>
<td>0</td>
<td>Mild restlessness, erection of hair coat, increased respiratory rate in the first 5 minutes, confusion, scratching of nostrils and head, anorexia, sensitive to slight sound, moving around restlessly and whipping of their mouths.</td>
</tr>
<tr>
<td>B</td>
<td>5000</td>
<td>0</td>
<td>Moderate behavioral changes as seen in A.</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant part</th>
<th>% inhibition (mm) at varying concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(mg/ml)</td>
</tr>
<tr>
<td>October – December</td>
<td>2010</td>
</tr>
</tbody>
</table>
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*

<table>
<thead>
<tr>
<th>Plant part</th>
<th>% inhibition (mm) at varying concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(mg/ml)</td>
</tr>
<tr>
<td>Leaves</td>
<td>36</td>
</tr>
<tr>
<td>Pulp</td>
<td>49</td>
</tr>
<tr>
<td>Stem bark</td>
<td>31</td>
</tr>
<tr>
<td>Fulcin</td>
<td>90</td>
</tr>
<tr>
<td>SDA</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant part</th>
<th>% inhibition (mm) at varying concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5(mg/ml)</td>
</tr>
<tr>
<td>Leaves</td>
<td>39</td>
</tr>
<tr>
<td>Pulp</td>
<td>0</td>
</tr>
<tr>
<td>Stem bark</td>
<td>0</td>
</tr>
<tr>
<td>Fulcin</td>
<td>86</td>
</tr>
<tr>
<td>SDA</td>
<td>0</td>
</tr>
</tbody>
</table>

**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


