Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles

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Abstract

In the indigenous health care delivery system of Ethiopia, numerous plant species are used to treat diseases of infectious origin. Regardless of the number of species, if any of such claims could be verified scientifically, the potential significance for the improvement of the health care services would be substantial. The objective of this study was, therefore, to determine the presence of anti-microbial activity in the crude extracts of some of the commonly used medicinal plants as well as to identify the class of compounds in the plants that were subjected to such screening. Thus, the crude methanol, petroleum ether and aqueous extracts of 67 plant species were subjected to preliminary screening against 10 strains of bacterial species and 6 fungal strains using the agar dilution method. A sample concentration of 250–2000 μg/ml and 500–4000 μg/ml were used for the bacterial and fungal pathogens, respectively. The results indicated that 44 different plant species exhibited activity against one or more of the bacteria while one species, viz., Albizzia gummifera showed activity against all the 10 bacteria at different gradient of dilution. Twenty three species inhibited or retarded growth of one or more organisms at dilution as low as 250 μg/ml. Extracts of same plants species were also tested against six different fungal pathogenic agents of which eight species showed growth inhibition against one or more of the organisms. Trichilia emetica and Dovyalis abyssinica, which inhibited growth of four and five fungal strains at 100 μg/ml concentration, respectively, were the most promising plants. Chemical screening conducted on the extracts of all the plants showed the presence of several secondary metabolites, mainly, polyphenols, alkaloids, tannins, sterols/terpenes, saponins and glycosides. The plants containing more of these metabolites demonstrated stronger anti-microbial properties stressing the need for further investigations using fractionated extracts and purified chemical components.

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Keywords: Anti-microbial activity; Phytochemical screening; Medicinal plants; Extracts

1. Introduction

For centuries, most of the population in Ethiopia, as elsewhere in many other developing countries, have relied on a system of traditional medicine, which consists of both empirico-rational and magico-religious elements or at times a combination of both. Infectious diseases, which account for the significant proportion of the health problems, are most often catered for by this system of medicine. Whether the approach employed is empirico-rational or magico-religious, plants constitute the centre-piece of therapy in this system of medicine for restoring or maintaining well-being of the people.

Pharmacological and phytochemical insights into several plants that were similarly used in other countries have led either to the isolation of novel structures for the manufacture of new drugs or to templates that served for the production of synthetically improved therapeutic agent. For example, of the...
104 new drugs developed over 37 years, 60 originated from plants used in traditional medicine of China (Gen, 1986). Furthermore, the discovery of modern drugs such as quinine, vincristine, digoxin and digitoxin, emetine, artemisinine, etc., from medicinal plants signify the huge potential that still exists for the production of many more novel pharmaceuticals (Plotkin, 1988). Thus, there has recently been a resurgence of interest in the development of drugs from the plants, especially from those of the developing countries that have a rich heritage of botanical ethnopharmacopoea.

In view of this, the search for new anti-microbial agents from medicinal plants is even more urgent in the context of countries like Ethiopia where infectious diseases of bacterial and fungal origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics (Hart and Karriuri, 1998; Abbe et al., 2003). Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products from plants used in folklore medicine is further justified. As wide spread as infectious diseases are in Ethiopia, the number of medicinal plant species prescribed against infectious diseases runs into the hundreds.

Many of these plants, of course, have a prolonged and uneventful use that may serve as indirect testimony to their efficacy. However, in the absence of objective proof of efficacy and without the knowledge of the constituents responsible for the stated physiologic actions, the validity of the remedies is questionable and their use would remain locally restricted. Previous studies, albeit few, have attempted to shade light on the anti-microbial activities of some indigenous medicinal plants (Desta, 1993; Askehr and Ashenafi, 1999a, 1999b; Lemma et al., 2002). Nonetheless, the medicinal flora of the country still remains virtually unexplored from the point of view of biological/pharmacological activity hampering their broader utilization in the official health care delivery system, particularly at the PHC level.

The purpose of this study was, therefore, to carry out preclinical evaluation of some popular medicinal plant species, i.e., biological and physiochemical screening with particular emphasis on those that seem to have very little or no scientific information in the areas intended for the investigation. It is also hoped that the study will facilitate the selection, for further investigation, of plants with relatively high level of potency and/or with wide range of biological activities. In the present study, 67 plant species were selected on the basis of the available ethnomedical information and were screened for their anti-microbial properties.

It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such as tannins, terpenoids, alkaloids, polyphenols are generally superior in their anti-microbial activities (Cowan, 1999). This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents. Therefore, simultaneous determination of the compounds that are possibly responsible for any biological activity would, inter alia, facilitate decision-making process as in the selection of the plants for in-depth future investigation. In view of this, we have also undertaken chemical screening of all the plants that were subjected to the biological screening.

2. Materials and methods

2.1. Plant material

2.1.1. Collection and identification

The plants or parts thereof used in this study were collected between February 2000 and May 2002 from several sites of Ethiopia in the wild at an altitudinal range of 900–3900 m. They were identified by a taxonomist using standard Floras, and voucher specimens were deposited in the Herbarium of the Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Addis Ababa.

2.1.2. Extract preparation

Air dried and powdered plant materials (0.5–1 kg) were extracted by maceration and percolation with 80% methanol or in some cases with 80% ethanol, petroleum ether (40–60 °C) and water at room temperature. The extracts were then filtered and concentrated under vacuum in rotary evaporator to give (as a percentage of powdered plant material) 2–11% gummy residue. All the extracts were kept in tightly stopped bottle in a refrigerator until used for the anti-microbial testing.

2.1.3. Phytochemical screening

Following botanical identification of the selected plants, total extracts were prepared from the freshly collected materials for the intended array of biological test systems. Portions of the same extract that was subjected for the biological screening was used for the identification of the major secondary metabolites employing the methodology outlined by Fransworth (1966), Marini-Bettolo et al. (1981); Harborne, (1973, 1984).

2.2. Test organisms

2.2.1. Bacterial strains

The standard organisms used as reference strains for the antibacterial tests were obtained from American Type Culture Collection or reference strains: Staphylococcus aureus (ATCC 27853), Streptococcus pyogenes (ATCC 19615), Streptococcus pneumoniae (ATCC 49619), Neisseria gonorrhoea (ATCC 49226) and Escherichia coli (ATCC 25922).

Other test organisms were isolated from patient samples of the Clinical Bacteriology Laboratory at the EHNRI. These were, Bacillus cereus, Shigella dysenteriae A, Shigella flexneri B, Salmonella typhi and Salmonella typhimurium bearing clinical isolate identification numbers 19618, 5264, 330605, 5266 and 5269, respectively.
<table>
<thead>
<tr>
<th>Collection number</th>
<th>Species (vernacular name)</th>
<th>Plant part</th>
<th>Solvent</th>
<th>Class of compounds</th>
<th>Concentration of extracts</th>
<th>Traditional medical use</th>
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</thead>
<tbody>
<tr>
<td>LA-2036</td>
<td>Laggera alata (D.Don) Sch.Bip (Compositae)</td>
<td>Kesebedeje</td>
<td>MeOH</td>
<td>D, E</td>
<td>1</td>
<td>Cgh</td>
</tr>
<tr>
<td>CH-2022</td>
<td>Clematitis lanata Pers. &amp; Guill. (Ranunculaceae)</td>
<td>Azu-hareg</td>
<td>MeOH</td>
<td>C, F</td>
<td>2</td>
<td>Wod</td>
</tr>
<tr>
<td>LF-2080</td>
<td>Laggera tomentosa Sch.Bip (Compositae)</td>
<td>Kokoso</td>
<td>MeOH</td>
<td>D, E</td>
<td>3, 4, 2, 3, 2</td>
<td>Rpg, Tec, Swi</td>
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<tr>
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<td>A, C, E</td>
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<td>Lep, Wod, Lif, Syp</td>
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<tr>
<td>AA-2131</td>
<td>Artenisia abyssinica Sch.Bip ex Rich (Compositae)</td>
<td>Chikugn</td>
<td>EtOH</td>
<td>C, E</td>
<td>5, 1, 2, 1, 2, 5*</td>
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<td>CA-2025</td>
<td>Clausena metani (Wild) Hook. f. ex Benth. (Rutaceae)</td>
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<td>Wod, Cgh</td>
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<td>SA-2033</td>
<td>Stephanus abysinica (Dill. &amp; Rich) Walp (Menispermaceae)</td>
<td>Ayit Hareg</td>
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<td>2</td>
<td>Anx, Rab, Syp, Dta, Maa</td>
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<td>Lobelia rhynchopetalum (Hochst.) Hemsl. (Lobiaceae)</td>
<td>Yedega-jibra</td>
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<td>Rab, Sch, Mls, Les</td>
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<td>LG-2046</td>
<td>Lobelia globosa Hemsl. (Lobiaceae)</td>
<td>Yekola-jibra</td>
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<td>AS-2035</td>
<td>Adhatoda zamppornia (Hochst.) Nee (Acanthaceae)</td>
<td>Senel</td>
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<td>Verbenacumin satimucum Benth. (Scrophulariaceae)</td>
<td>Yeahya joro</td>
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<td>Lep, Ant, Sch, Syp, Mls, Gms</td>
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<td>Euclea divinorum Hiern (Ebenaceae)</td>
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<td>Bucida abyssinica (A. Rich) Warburg. (Flacourtiaceae)</td>
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<td>Lep, Amb, Rab, Syp</td>
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<td>Ant, Les, Lep, Sch</td>
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<td>WU-2045</td>
<td>Warburgia spicata (Schum.) Canale (Canaleaceae)</td>
<td>Bilu</td>
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<td>C, D, E</td>
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<td>Bilu</td>
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<td>C, D, E</td>
<td>1, 2, 5, 8</td>
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Table 1: In vitro antimicrobial activity of traditionally used medicinal plant extracts.
Table 1 (Continued)

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<th>Collection number</th>
<th>Species Vernacular name</th>
<th>Plant part</th>
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<th>Class of compounds</th>
<th>Concentration of extracts 2000 μg/ml</th>
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<th>250 μg/ml</th>
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<td>ZS-2080</td>
<td>Zehneria acabra (L.) Sond. (Cucurbitaceae)</td>
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<td>D, E, F</td>
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<td>CP-2064</td>
<td>Cecropia aconitifolia Schinz. (Amaranthaceae)</td>
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<td>GA-2006</td>
<td>Albizia gummifera (JF.Gmel.) C.A.Sm. (Leguminoseae)</td>
<td>Ambabesa</td>
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<td>A, C, E, F, G</td>
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<td>Cordia africana Lam. (Boraginaceae)</td>
<td>Wanza</td>
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<td>AC-2070</td>
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<td>Pentas lanceolata (Forsk.) Delile (Rubiaceae)</td>
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<td>JA-2065</td>
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<td>Yikurumbe</td>
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Table 1 (Continued)

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<th>Code</th>
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<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Myrica salcifolia A. Rich</td>
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<td>E</td>
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<td>G</td>
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<td>Ovidiaeus aub. capitate (Wall. Ex DC.) (Oleaceae)</td>
<td>Woyera Lr</td>
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Extract effect: *, growth retarded. Plant part: Ar, aerial part; Rt, root; Fr, fruit; Sd, seed; Sb, stem bark; Lv, leaves; Wp, whole plant; St, stem; Rb, root bark.

3. Results

Activity evaluations of the extracts belonging to 44 species (66%) exhibited activity against one or more bacterial strains. The alcoholic (methanolic/ethanolic) extracts exhibited higher antibacterial effects than the corresponding petroleum ether extracts.

2.2.2. Fungal strains

Twenty three species inhibited or retarded growth of one or more fungal strains. The alcoholic (methanolic/ethanolic) extracts inhibited higher antibacterial effects than the corresponding petroleum ether extracts.

2.1. Standard antibiotics

The standard antibiotics used for the antifungal tests included Amphotericin (Lot No. 606106), Chloramphenicol (Lot No. 1006 B10540), Co-trimoxazole (Lot No. 3770), Gentamycin (Lot No. 1006-B12501), Kanamycin (Lot No. 1006-80354), Kanamycin (Lot No. 100-580167), Sulphadiazine (Lot No. 1005-803617), Tetracycline (Lot No. 1006-811596), Trimethoprim (Lot No. 1006-811598), Vancomycin (Lot No. 1006-803540), and Vibramycin (Lot No. 1006-803541). The standard reference isolates were obtained from the Clinical Bacteriology Laboratory of the Department of Microbiology, Faculty of Science, University of Addis Ababa.

Activity evaluation of the extracts belonging to 44 species (66%) exhibited activity against one or more bacterial strains. The extracts were also tested against some of the above bacterial strains.

3. Results

The antimicrobial tests were conducted using the agar dilution method. The standard strains included Amphotericin (Lot No. 606106), Chloramphenicol (Lot No. 1006 B10540), Co-trimoxazole (Lot No. 3770), Gentamycin (Lot No. 1006-B12501), Kanamycin (Lot No. 1006-80354), Kanamycin (Lot No. 100-580167), Sulphadiazine (Lot No. 1005-803617), Tetracycline (Lot No. 1006-811596), Trimethoprim (Lot No. 1006-811598), Vancomycin (Lot No. 1006-803540), and Vibramycin (Lot No. 1006-803541). The standard reference isolates were obtained from the Clinical Bacteriology Laboratory of the Department of Microbiology, Faculty of Science, University of Addis Ababa.

Activity evaluation of the extracts belonging to 44 species (66%) exhibited activity against one or more bacterial strains. The extracts were also tested against some of the above bacterial strains.
Table 2

<table>
<thead>
<tr>
<th>Collection number</th>
<th>Species Vernacular name</th>
<th>Parts used</th>
<th>Solvent</th>
<th>Class of compound</th>
<th>Concentration of extracts</th>
<th>Traditional medical use</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE-2041</td>
<td>Trichila emetica Vahl (Melianthaceae)</td>
<td>Roka Fr</td>
<td>MeOH</td>
<td>C, D, E</td>
<td>1, 2, 3, 5, 6</td>
<td>Mal, Ane</td>
</tr>
<tr>
<td>DV-2063</td>
<td>Dovyalis abyssinica (A.Rich) Warburg (Flacourtiaceae)</td>
<td>Koshim Lv</td>
<td>MeOH</td>
<td>C, D, E, F</td>
<td>1, 2, 3, 4, 5, 6</td>
<td>Tap, Toa, Sot</td>
</tr>
<tr>
<td>XA-2052</td>
<td>Ximenia americana L. (Olacaceae)</td>
<td>Enkoy Lv</td>
<td>MeOH</td>
<td>C, D, F</td>
<td>1*, 2*</td>
<td>Amb, Gon, Vim, Rab, Sot</td>
</tr>
<tr>
<td>AG-2006</td>
<td>Albizia gummifera (JF.Gmel) C.A.Sm. (Leguminaseae)</td>
<td>Ambabesa Sd</td>
<td>MeOH</td>
<td>C, E, F, G</td>
<td>2, 5*, 6*</td>
<td>Scb</td>
</tr>
<tr>
<td>SG-2059</td>
<td>Syzygeum guineense (Wild.) DC. (Myrtaceae)</td>
<td>Dokma Lv</td>
<td>MeOH</td>
<td>C, D, E</td>
<td>1*, 2*</td>
<td>–</td>
</tr>
<tr>
<td>ES-2053</td>
<td>Euclea divinorum Hiern (Ebenaceae)</td>
<td>Dedeho Lv</td>
<td>MeOH</td>
<td>C, D, E</td>
<td>1*, 2*</td>
<td>–</td>
</tr>
<tr>
<td>OQ-2044</td>
<td>Osyris quadripartite Decn. (Santalaceae)</td>
<td>Keret Lv</td>
<td>MeOH</td>
<td>C, E</td>
<td>1*, 2*, 3*, 4*, 5*, 6*</td>
<td>Ant, Les, Lep, Scb</td>
</tr>
<tr>
<td>GL-2024</td>
<td>Gardenia lutea Fres. (Rubiaceae)</td>
<td>Gambilo Sb</td>
<td>MeOH</td>
<td>C, E</td>
<td>2*, 5*</td>
<td>–</td>
</tr>
</tbody>
</table>

Organisms: 1, Candida albicans; 2, Cryptococcus neoformans; 3, Aspergillus flavus; 4, Aspergillus niger; 5, Tricophyton mentagrophytes; 6, Tricophyton violacium. Extract effect: *, growth retarded.

The anti-microbial activity of the extracts of the various parts of the investigated plants such as roots, leaves, fruits, seeds, etc. appears to be due to the presence of secondary metabolites such as polyphenols identified in 92% of the plant species, triterpenes/sterols (83%), saponins (28%), tannins (14%), alkaloids (44%), glycosides and polysaccharides (11%) while cardiac glycosides were identified in few species (Table 1).

Plants, which accumulate polyphenols, tannins and unsaturated sterols/terpenes were shown to inhibit or significantly retard growth of eight of the ten test organisms. The species, which constitute polyphenols and unsaturated sterols/terpenes; and polyphenols, tannins, unsaturated sterols/terpenes, saponins and glycosides inhibited six organisms each while those with polyphenols, tannins, unsaturated sterols/terpenes, saponins, and alkaloids and unsaturated sterols/terpenes inhibited growth of five bacterial strains each.

Fungicidal and/or growth retardant activity was demonstrated by the extracts of only eight species out of 63 plants at dilution ranges of 500–4000 μg/ml (Table 2). Trichila emetica and Dovyalis abyssinica, which inhibited growth of four and five fungal strains at 1000 μg/ml concentration, respectively, were the most potent and the more promising taxa for further in-depth investigations.

4. Discussion

Infectious disease of microbial origin, such as Neisseria gonorrhoea, Staphylococcus aureus, Bacillus cereus, Shigella spp., etc., constitute the major cause of morbidity and/or mortality in countries like Ethiopia (Kloos and Zein, 1993). With the emergence of HIV, the negative role of these micro-flora has even become worse as they facilitate the infection rate by the virus or by significantly reducing the onset time of AIDS. Nowadays, there are very few, if any, antibiotics to which these micro-organisms have not developed resistance.

The situation is further compounded by the lack of patient compliance to antibiotic regimen and by the exorbitant costs of the antibiotics. The preliminary results of the present study, therefore, not only confirms the justifiable use of some of the plants against these micro-organisms in the traditional health care system but also reflects the hope for development of effective chemotherapeutic agents in the future from same or similar plants.

It generally appears that the more the constituent in a given species, the more diverse the micro-organisms it acts upon. This was substantiated by the fact that 20...
species (ca. 47%), which accumulate 2–5 compounds, in-
hibited three or more organisms compared to 19 species
(44%) with 2–3 compounds that inhibited growth of only
one type of organism. Until further bioassay guided frac-
tionation is undertaken to determine the most active con-
stituent, the likely explanation to this phenomena could,
therefore, be the more the number of compounds in
the total extract the better the activity possibly due to
synergism.

The importance of fungicidal activity investigation can
not be over emphasized in view of the fact that, fungal
infections of the skin, nails and hair are a major source
of morbidity through out the world, accounting for about
20% of new out patient referrals in the tropical countries
like Ethiopia where the damp and humid climatic condi-
tions coupled with the advent of HIV/AIDS infection tend
to aggravate skin disorders of fungal origin (Abebe et al.,
2003).

The activity of some of the plant extracts on different or-
ganisms explains their broad spectrum nature while most of
the plant extracts found to have effect on one organism may
be due to their narrow spectrum of activity. This difference
of activity appears to be directly related to the qualitative
and/or quantitative diversity of the compounds that are be-
ing accumulated by the plants investigated. Though, the MIC
of the plant extracts are no match to those of the standard
antibiotics, it is hoped that they might produce comparable
effect on further purifications and/or isolation of the active
constituents.

The fairly good degree of correlation of traditional
therapeutic claims with the specific anti-microbial activ-
ity as observed in the present preliminary results war-
rant further investigation. Thus, activity guided fractiona-
tion of the constituents of the most promising plants as
well as acute toxicity studies are already underway in our
laboratory.

Acknowledgements

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tion Research Institute for financial support.

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