Traditional dietary additives of the Maasai are antiviral against the measles virus

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Abstract

Although ethnopharmacological investigations emphasize the importance of medicinal plants in developing countries, species used regularly with diet are under-investigated and potentially make greater contributions to health. Thirteen traditional plants most commonly added to milk/soups by the Maasai for perceived health benefits were tested for activity against measles virus (MV) using non-medicinal plants as controls. Antiviral effects of plant extracts were sought using a modified neutralization assay. Methanolic extracts of medicinal species exhibited significantly greater activity neutralizing MV in vitro in comparison to non-medicinal extracts (p < 0.02). Four of 13 (31%) medicinal species versus 0/13 controls had measurable effects against MV in vitro. Olinia rochetiana (Olkirenyi) and Warburgia ugandensis (Osokonoi) extracts were most potent with the number of plaque forming units reduced 37- and 34-fold, respectively. Given the importance of monocytes in the dissemination of MV, we assessed the capacity of a subset of plant extracts to inhibit MV growth in monocytoid cell line, U937. MV output from U937 cells was significantly reduced by four of seven medicinal plant extracts (mean reduction 48 h: 39.0 ± 26.0%, range 3.5–87%; 72 h: 56.4 ± 29.5%, range 14.1–103.1%) (p < 0.05).

This study provides evidence that medicinal plants added to the Maasai diet may contribute to the modulation of viral infections.

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1. Introduction

Local remedies for various ailments have a long history of use in rural Africa and traditional medicines that have antimicrobial activity could have important benefits in communities where they are widely used. As traditional knowledge of disease treatment erodes in the face of socioeconomic change, the urgency to document indigenous remedies increases (Farnsworth, 1993).

Abbreviations: C, centigrade; CDC, Centre for Disease Control; CQ, chloroquine; CPM, counts per minutes; CO2, carbon dioxide; DMSO, dimethyl-sulphoxide; DNA, deoxyribonucleic acid; FCS, fetal calf serum; HPLC, high-performance liquid chromatography; IC, inhibitory concentration; KEN-RIK, Kenya Resource Centre for Indigenous Knowledge; ml, millilitre; MeOH, methanol; MV, measles virus; MOI, multiplicity of infection; μCi, microcurie; μl, microlitre; NV, neutralization value; pfu, plaque forming units; PRN, plaque reduction neutralization; RNA, ribonucleic acid; RT, room temperature

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Maasai pastoralists of East Africa incorporate plant species into the diets of both healthy and sick children, often in combination with milk and soup (Johns et al., 1999; Johns, 1999a,b). Although ethnopharmacological investigations have historically emphasized the targeted use of medicinal plants (i.e. for therapy), species used in conjunction with diet are under-investigated despite their potentially greater contributions to health. This study evaluates the Maasai method of coping with measles and diet-based practices used to maintain child health. Although the majority of plants reported did have symptom specific indications reported by traditional healers, mothers generally employed these same plants on a regular basis to prevent sickness by bringing medicine to the diet using soups, milk, and teas.

Measles is among the most serious of the common childhood diseases. Each year, measles virus (MV) infects approximately 40 million children and causes the death of approximately 500,000 infected individuals worldwide (Arvin, 2000). MV enters the human body via the respiratory tract. After primary
infection in the respiratory epithelium, MV spreads throughout the body, infecting immune cells as well as a wide range of endothelial and epithelial tissues (Griffin and Bellini, 1996; Chabot and Ward, 2002). Monocytes are a major target for MV infection in the blood and may contribute to viral dissemination (Esolen et al., 1993). Antiviral agents that inhibit viral replication within monocytes may therefore be particularly beneficial in modulating the severity of disease. At the current time, only high dose vitamin A has been shown to have any therapeutic benefit in acute MV infection (Fawzi et al., 1993; Glasziou and Mackerras, 1993). None of the available antiviral drug classes has any significant activity against this virus. Although antiviral MV activity has been reported in various East African plants used therapeutically, we are not aware of any effort to follow up these preliminary observations (Vlietinck et al., 1995; Sindambwiwe et al., 1999; Cos et al., 2002).

This study hypothesized that extracts of routinely used medicinal plants would exhibit activity against MV that would not be present in extracts from arbitrarily selected non-medicinal plants. Plant extracts were screened using a modified plaque reduction neutralization assay and potential antiviral activity was further assessed in a subset using MV-infected U937 (monocytic) cells. Potential toxicity of the plant extracts was assessed by viability and proliferation assays.

2. Methods and materials

2.1. Plant collection

Plants were collected within the Loita Hills region, Narok District, Kenya, during July and August of 2002. Medicinal plant use was surveyed during 50 randomized dietary interviews in different villages within the six subcentres of Loita, Ilkerin, Morijo, Olmesuti, Entasekera, Olngrua and Olorte (Parker, 2004). When interviewed, mothers were asked to report the species they would employ to treat child measles infection. Mothers consistently responded that they used an array of plants to promote and maintain child health on a daily basis regardless of sick or healthy status. However, traditional healers reported certain species within this generic array of plants were believed to be helpful in remedying symptoms common to most sicknesses (e.g. fever, stomach ache, and red eyes). The 13 dietary medicinal plants most frequently reported as given to children in milk or soups were collected as well as 13 arbitrarily selected non-medicinal species. The non-medicinal species selected had a variety of uses including firewood, construction, ceremonial purposes, and sometimes no use was reported. Voucher specimens were identified by Patrick Maundu, Ruth Adeka, and Joshua Muasya of Kenya Resource Centre for Indigenous Knowledge (KENRIK) and deposited at the East African Herbarium, National Museums of Kenya, Nairobi.

2.2. Extraction of natural product

All species selected for analyses were listed with their Maasai and scientific names in Table 1. Dried plant material was ground using a Wiley mill with an 850 μm sieve. Samples were extracted with methanol (HPLC grade) using the Soxtect HT extraction system and dried under vacuum (Rotavapor). The residue was then freeze dried for 24 h and crystals were collected for storage in −80 °C freezer.

2.3. Plaque neutralization assay

To assess direct effects of plant extracts on MV growth in epithelial cells (Vero cells), we modified the plaque reduction neutralization (PRN) assay that is commonly used to measure anti-measles antibodies (Albrecht et al., 1981). Briefly, serial dilutions of extracts starting at 1 μg/ml and appropriate DMSO controls were mixed and incubated with ~25 plaque forming units (pfu) of low-passage Edmonston vaccine-strain MV for 90 min at 37 °C in 100 μl of Hanks buffered saline solution containing magnesium and calcium (Wisent). At the end of incubation, virus:extract mixtures were placed in duplicate on 85–90% confluent Vero cell monolayers in 24-well plates (Falcon, BD Biosciences, Mississauga, Ontario, Canada) in tenfold serial dilutions (100 μl/well). Plates were incubated for 90 min at 37 °C in 5% CO2 to permit viral attachment and entry. Residual unattached virus was aspirated from each well and a 16% methylcellulose overlay in Liebovitz’s L-15 media (Gibco/Life Technologies, Grand Island, NY) was applied (1 ml/well). After an additional 4 days incubation at 37 °C in 5% CO2, 500 μl/well of 4% neutral red was added and the staining of the monolayers occurred over 24 h at room temperature (RT). Finally, cell monolayers were fixed with 3.7% formalin for 10 min and visible plaques were counted manually. A high-titer human serum served as positive control. A neutralization value (NV) for each extract was obtained using the Kaber method (Ward et al., 1999). NVs are expressed as the log2 of the reciprocal of extract dilution calculated to reduce the number of plaques by 50%.

2.4. Impact of plant extracts on MV output in U937 cells

Seven medicinal and seven non-medicinal plant extracts were evaluated using this assay (identified in Fig. 1). The seven most frequently used medicinal plants were chosen while non-medicinal plants were randomly selected from the originally arbitrarily selected species. To test the effects of plant extract on MV growth in monocytes, a U937 cell line was used. U937 cells were used as they are already known to support measles replication and serve as a model for human monocytes since this is a human myelomonocytic cell line (Helin et al., 1999). U937 cells were grown in RPMI 1640 medium with 10% fetal calf serum (FCS Wisent), 1% Heps (Wisent), and 0.01% gentamicin (Wisent). At a multiplicity of infection of 1 (MOI = 1), 1 × 106 cells/ml were infected with a tissue culture-adapted MV strain (Chicago-1 kindly provided by W Bellini, CDC, Atlanta). Virus was permitted to attach for 90 min at 37 °C in 5% CO2. Following infection, U937 cells were centrifuged (200 × g for 10 min) to remove unattached virus and resuspended in RPMI medium containing 2% FCS at a density of 1 × 105 cells/ml in 24-well plates (1 ml/well). Serial dilutions of each plant extract (500 μl) were added to MV-infected U937 cells in duplicate wells and incubated for 72 h. Aliquots of cells (200 μl) were
Table 1
Traditional Maasai plants chosen for analysis of potential antiviral activity

<table>
<thead>
<tr>
<th>Medicinal plants (family) voucher number</th>
<th>Maasai name</th>
<th>Plant part and use</th>
<th>Households reported using per week (%)</th>
<th>Assay</th>
<th>Non-medicinal plants (family) voucher number</th>
<th>Maasai name</th>
<th>Plant part used</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhus natalensis Bernh. Et Krauss (Anacardiaceae), 6</td>
<td>Olmisigiyoi</td>
<td>SB milk tea</td>
<td>76</td>
<td>1, 2</td>
<td>Apodytes dimidiatu E. Mey. Ex Arn. (Icacinaceae), 40</td>
<td>Oloiondo</td>
<td>SB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Olinia rochetiana (Oliniaceae), 11</td>
<td>Olkirenyi</td>
<td>SB milk</td>
<td>74</td>
<td>1, 2</td>
<td>Buddleja polystachya Fres. (Buddleiacae), 66</td>
<td>Olpiron</td>
<td>SB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Rhamnus prinoides L’Hér. (Rhamnaceae), 27</td>
<td>Olkonyil</td>
<td>RB milk soup</td>
<td>72</td>
<td>1, 2</td>
<td>Dombeya rotundifolia (Hochst.) Planch. (Sterculiaceae), 33</td>
<td>Oltotoo</td>
<td>SB</td>
<td>2</td>
</tr>
<tr>
<td>Scutia myrtina (Burm.f.) Kurz (Rhamnaceae), 21</td>
<td>Osanangururi</td>
<td>SB soup</td>
<td>70</td>
<td>1, 2</td>
<td>Erythrina abyssinica Lam. (Fabaceae), 30</td>
<td>Olopoli</td>
<td>SB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Trimeria grandifolia (Hochst.) Warb. (Flacourtiaceae), 26</td>
<td>Oledat</td>
<td>RB soup</td>
<td>66</td>
<td>1, 2</td>
<td>Ficus thornningi Blume (Moraceae), 42</td>
<td>Olitiasimbol</td>
<td>SB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Ximenia americana L.; Ximenia caffra Sond. (Olacaceae), 19</td>
<td>Olamai</td>
<td>SB soup</td>
<td>48</td>
<td>1, 2</td>
<td>Indigefera trita L. f. (Fabaceae), 60</td>
<td>Enkoroi</td>
<td>SB</td>
<td>2</td>
</tr>
<tr>
<td>Acacia nilotica (L.) Willd. Ex Delile (Fabaceae), 17</td>
<td>Olkilori</td>
<td>SB soup</td>
<td>42</td>
<td>1, 2</td>
<td>Juniperus procera Hochst. Ex Endl. (Cupressaceae), 48</td>
<td>Oltarakwai</td>
<td>SB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Toddalia asiatica (L.) Lam. (Rutaceae), 56</td>
<td>Oleparumuno</td>
<td>RB</td>
<td>40</td>
<td>2</td>
<td>Mystroxylon aethiopicum (Thunb.) Loes. (Celastraceae), 50</td>
<td>Olodonganayo</td>
<td>SB</td>
<td>2</td>
</tr>
<tr>
<td>Albizia amara (Roxb.) Boivin (Fabaceae), 8</td>
<td>Olperelengo</td>
<td>SB</td>
<td>40</td>
<td>2</td>
<td>Occhna holstii (Ochnaeaceae), 44</td>
<td>Olcharuyan</td>
<td>SB</td>
<td>2</td>
</tr>
<tr>
<td>Warburgia ugandensis Sprague (Canellaceae), 58</td>
<td>Osokonoi</td>
<td>SB</td>
<td>34</td>
<td>2</td>
<td>Podocarpus falcatus (Podocarpaceae), 62</td>
<td>Olpiripiri</td>
<td>RB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Osyris abyssinica A. Rich. (Santalaceae), 68</td>
<td>Olosesiai</td>
<td>SB</td>
<td>30</td>
<td>2</td>
<td>Podocarpus falcatus (Podocarpaceae), 62</td>
<td>Olpiripiri</td>
<td>SB</td>
<td>2</td>
</tr>
<tr>
<td>Plumbago zeylanica L. (Plumbaginaceae), 72</td>
<td>Olgeriandus</td>
<td>RB</td>
<td>30</td>
<td>2</td>
<td>Solanum manense Bitter (Solanaceae), 70</td>
<td>Endemelu</td>
<td>RB</td>
<td>1, 2</td>
</tr>
<tr>
<td>Oncocalyx fischeri (Loranthaceae), 31</td>
<td>Entaretoi</td>
<td>Stems, leaves</td>
<td>24</td>
<td>2</td>
<td>Vangueria apiculata K. Schum. (Rubiaceae), 36</td>
<td>Ilgumi</td>
<td>Stems, leaves</td>
<td>2</td>
</tr>
</tbody>
</table>

Medicinal plants are listed in order of reported frequency of use by children within 50 households surveyed, with plant part used and mode of consumption. (SB) Stem bark; (RB) root bark; assays: (1) plaque assay (2) plaque reduction neutralization.
removed from each well following brief titration at 24, 48, and 72 h. Aliquots were subjected to a freeze–thaw cycle and the level of MV replication was quantified by plaque assay (as above without the plant extracts pre-incubation).

2.5. Toxicity of extracts for cell lines used

Cell viability was assessed by 0.01% trypan blue dye exclusion after 24 and 72 h exposure to each plant extract at 37 °C in 5%CO₂ over a range of concentrations (0.001–10 μg/ml). At each time point, the total numbers of viable and dead cells were counted by hemacytometer. Since actively proliferating cells generally support better MV replication than resting cells, the impact of plant extracts on U937 proliferation was measured by 3H-thymidine incorporation as previously described (Ward et al., 1995). Briefly, U937 cells were seeded in triplicate into 96-well plates at a density of 50,000 cells/well in RPMI medium (Wisent) containing 10% fetal bovine serum (Wisent) and 3H-thymidine (1 μCi per well) and serial dilutions of plant extracts over a range of concentrations (0.001–10 μg/ml final concentration). U937 cells were incubated at 37 °C and 5% CO₂ for 16 h and subjected to one freeze–thaw cycle before cellular DNA was harvested onto glass-fiber filters, and thymidine incorporation was measured by beta-counter (Microbeta, Wallac, Finland). The results are expressed as mean counts per minute (CPM) of triplicate samples.

2.6. Statistical analysis

In order to determine if medicinal plants exhibited greater antiviral activity than non-medicinal plant extracts in the modified PRN assay, a paired t-test was conducted to compare neutralization values. To determine if a greater proportion of medicinal plants had antiviral effects than non-medicinal plants in the plaque assay experiment, a one-tailed Fisher’s exact test was employed. For both tests, the significance level was set at 0.05.

3. Results

3.1. Extracts were not toxic at concentrations ≤1 μg/ml

At the highest concentrations tested (10 μg/ml), residual DMSO (20 μl/ml) treatment of Vero and U937 cells resulted in cell death suggesting that DMSO was toxic to both cell types tested (data not shown). At concentrations of 1 μg/ml and below, the DMSO control had no impact on either cell viability or proliferation. With the exception of the non-medicinal plant extract *Juniperus procera* (Oltarakwai) at 1 μg/ml in the proliferation assay, there was no evidence of toxicity for either Vero or U937 cells attributable to any of the other extracts at concentrations ≤1 μg/ml (data not shown).

3.2. Neutralization of measles by plant extracts

Four of the 13 (31%) medicinal plant extracts had measurable neutralizing effects on MV when incubated for 90 min at 37 °C prior to infecting Vero cell monolayers (Fig. 1A). In contrast, the non-medicinal plant extracts had no detectable neutralizing activity (Fig. 1B). The difference between individual neutralization values of the 13 medicinal and 13 non-medicinal species was statistically significant (t-test *p* < 0.02). Two extracts in particular, *Olinia rochetiana* (Olkirenyi) and *Warburgia ugandensis* (Osokonoi), had striking ability to neutralize virus during pre-incubation with calculated NVs of 107.3 and 98.0, respectively. These values suggest that as little as 0.1 μg/ml of these extracts can neutralize 50% of the input virus particles. The positive human serum control included in these assays had a NV of 293.5.

3.3. Effects of plant extracts on MV viral output by U937 cells

None of the non-medicinal species decreased MV replication below control levels between 48 and 72 h in infected U937 cells. In contrast, four of the medicinal plant extracts, including one of the two extracts with activity in the neutralization assay, significantly decreased MV output from infected U937 cells between 48 and 72 h (*Olinia rochetiana*, and *Rhamnus prinoides* (Olkonyil), *Rhus natalensis* (Olmisigiyoi) and *Scutia myrtina* (Osanangururi) (Fig. 2). A greater proportion of medicinal extracts exhibited antiviral activity than non-medicinal extracts.
(one-tailed Fisher’s exact test \( p < 0.05 \)). At doses between 0.01 and 1 \( \mu \)g/ml, the mean suppression of MV output at 48 h was 39.0 ± 26.0% and ranged between 3.5 and 87%. At 72 h the mean reduction was 56.4 ± 29.5% and ranged from 14.1 to 103.1%. \textit{Rhus natalensis} and \textit{Rhamnus prinoides} were the most potent extracts at 48 h, exhibiting clear dose-dependent suppression while \textit{Olinia rochetiana} was most effective at 72 h in culture (Fig. 2).

4. Discussion

4.1. Viral neutralization

Most studies examining the antiviral capabilities of plant extracts against MV have been based on 50% suppression in plaque reduction neutralization (PRN) assays (Vanden Berghe and Vlietinck, 1991). In this study, the same approach was used. Five of 13 medicinal plant extracts (versus 0/13 non-medicinal) exhibited some degree of activity against MV in one or both of the assays used. Compared to mock control values, both \textit{Rhus natalensis} and \textit{Albizia amara} (Olperelengo) demonstrated 17-fold increases in MV neutralization. Even greater effects were seen with \textit{Olinia rochetiana} (37-fold increase), and \textit{Warburgia ugandensis} (34-fold). All four plant extracts should therefore be considered potential antiviral agents. The elucidation of the mechanisms underlying these antiviral effects on MV was beyond the scope of this study. However, due to the performance characteristics of the PRN assay, one may speculate that potential sites of activity may include inhibition of virus binding and/or entry which can be mediated by a number of cellular receptors such as CD46 present on Vero cells (Bellini et al., 1994). Direct lysis of the MV envelope and agglutination of virus particles by modulating sugar structures on interacting molecules (Griffin and Bellini, 1996) may also contribute to the antiviral activity of \textit{Rhus natalensis}, \textit{Albizia amara}, \textit{Olinia rochetiana} and \textit{Warburgia ugandensis}. It is also likely that different mechanisms or combinations of mechanisms participate in the antiviral activities of the different plant extracts.

Results of this study provide further evidence for the antiviral activity of \textit{Rhus natalensis}. In Maasai culture, the name Olmisigiyoi is used interchangeably for both \textit{Rhus natalensis} and \textit{Rhus vulgaris} because they are considered identical. Vlietinck et al. (1995) have previously shown in a study investigating the antiviral potential of 100 East African plants that \textit{Rhus vulgaris} root extract had potent antiviral activity against MV. \textit{Rhus natalensis} has also been found to confer protection from periodontopathic bacteria, possibly by inhibiting bacterial enzyme function (Homer et al., 1990, 1992).

For the first time, however, our results show that \textit{Warburgia ugandensis}, \textit{Albizia amara}, and \textit{Olinia rochetiana} extracts have antiviral activity against MV. In the case of \textit{Warburgia ugandensis}, when previously tested \textit{in vitro}, petroleum ether, ethanol, and water extracts did not have antiviral activity against MV (Olila et al., 2002). Aqueous MeOH and water extracts, however, have been found to exhibit antimicrobial, antibacterial and antifungal activity (Kubo and Taniguchi, 1988; Olila et al., 2001). Activity in MeOH extracts may be related to the presence of sesquiterpene dialdehydes (e.g. polygodial, warburganal, and muzigadial) known to have antibiotic activity (Kubo and Taniguchi, 1988). One sesquiterpene dialdehyde, polygodial, exhibited potent fungicidal activity by damaging cell membranes thereby facilitating the transmembrane transport of other chemicals into cells (Kubo and Taniguchi, 1988). MeOH extracts of \textit{Albizia amara} enabled the isolation of four macrocyclic pithecolobine alkaloids, with one isolate consisting of a mixture of budmunchiamines capable of interacting with DNA and inhibiting both DNA and RNA polymerases (Mar et al., 1991; Pezzuto et al., 1991). Finally, there is no evidence in the literature of prior work with \textit{Olinia rochetiana}. The antiviral potential of \textit{Olinia rochetiana} may therefore be reported here for the first time. Although \textit{Erythrina abyssinica} has previously been reported to have activity against MV \textit{in vitro} (Vlietinck et al., 1995), our study could not confirm these findings.
4.2. Inhibition of viral replication

*Olinia rochetiana* and *Rhus natalensis* extracts were active both in the PRN assay and in reducing viral replication in U937 cells. In contrast, *Rhamnus prinoides* and *Scutia myrtina* reduced MV output from infected U937 cells but had no activity in the PRN assay. Since Vero cell monolayers were used to quantify the output from infected U937 cells, a trivial explanation for the *Olinia rochetiana* and *Rhus natalensis* findings would be neutralization of viral particles by residual extract in the culture supernatants. However, these were the only two extracts that had activity in both assays.

*Rhamnus prinoides* and *Scutia myrtina*, on the other hand, had no activity in the PRN assay and likely exerted their antiviral activity by inhibiting one of the stages of the viral replication cycle within the infected cells. At present, very little has been published on either *Rhamnus prinoides* or *Scutia myrtina* and no previous studies have investigated their antiviral potential. *Rhamnus prinoides* has been found to be a potent anti-malarial agent against chloroquine (CQ)-sensitive and resistant strains of *Plasmodium falciparum*. MeOH extracts made from root bark produced the most potent IC(50) value at 15.1 ± 2.3 µg/ml in comparison to other solvents used (Muregi et al., 2003).

Given that a methanol extraction process was used in the current study, it is likely that the active compounds are fat soluble and lipophilic in nature. This would infer an ability of components to cross host cell lipid bilayer membranes to act against viruses at the intracellular level. Such compounds might be particularly active against enveloped viruses such as measles that complete their entire life cycle in the cytoplasm of infected cells (e.g. replication, transcription, translation, post-translational modification, assembly and budding). Potentially active constituents derived from methanol extracts include anthocyanins, terpenoids, saponins, tannins, xanthoxyllines, totarol, quassioids, lactones, flavones, phenones, or polyphenols (Cowan, 1999). Further investigations are needed to determine the mechanism(s) of action of all of these extracts.

4.3. Implications for traditional treatments

Among the medicines identified by traditional Maasai healers in this study *Olinia rochetiana* (olkirenyi), *Rhamnus prinoides* (olkonyil), *Toddralia asiatica* (oleparmunyo), and *Acacia nilotica* (oltuletlolowaru) are prescribed to treat the symptoms of internal disease including stomachaches, fevers, and respiratory infections. Measles specific symptoms such as (1) cough, (2) red rash, and (3) sore eyes were also dealt with externally by (1) hot steam inhalation with *Lagenaria abyssinica* (oltuletlolowaru), (2) bathing child in the extracts of Aloe roots and water used for steam inhalation with *Lagenaria abyssinica* extracts) once cooled, and (3) squirting breastmilk into sore eyes to wash away infection.

In studies of traditional remedies, the objectives are generally (a) to understand how traditional communities maintain/secure good health and (b) to identify potentially useful compounds that can be exploited by larger populations. Given limitations of the methods used to assess antiviral activity of the plant extracts in the current work, the data presented must be interpreted with caution. For example, in Maasai culture, medicinal plants are added to boiled milk for children to consume on a daily basis. Plants are taken with milk (daily) or soup (weekly) and are often used in combination. The oral bioavailability of the extracts tested is not known. Although MV replicates extensively in the gut late in disease, it initially enters the body via the respiratory tract. Although the antiviral activity of plant extracts was tested *in vitro* in monocytoid cells (U937) and a canine kidney epithelial cell line (Vero), the activity of these extracts in gut and respiratory epithelial cells remains to be determined. Nevertheless, one of the main complications of acute measles is diarrheal disease. Since plant extracts are ingested via the gastro-intestinal tract, further investigations could pursue the potential contribution of these medicinal plants in combating diarrheal complication of measles. All of the medicinal species studied with the exception of *Oncocalyx fischeri* (Entaretoi) appear in major compilations of medicinal plants from the region (Kokwaro, 1976; Neuwinger, 2000).

4.4. Conclusions

This study provides evidence that may justify traditional healers’ choice of specific medicinal species for not only the treatment of measles infection as well as for general ‘day-to-day’ ethnobotanical practice. We show for the first time that several plant extracts used by the Masai to maintain health can have potent antiviral activity against MV. Future investigations would determine whether or not these extracts have general or specific antiviral activity and identify and characterize the active fractions. These findings also support the argument that, if traditional medicine plants were not efficacious, then this form of health care would not have developed into a primary means of treatment in many communities (Githinji and Kokwaro, 1993).

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