Preliminary Screening of Crude Extracts of Fagaropsis Angolensis for Anticancer Activity

Antony Letoyah Yiaile1,2, James Mucunu Mbaria2, Isaac Mpapulu Ole-Mapenay4, Mitchell Otieno Okumu2, Abdi Hussein Hadun2, Jared Misonge Onyancha3

1Department of Pharmacy, University Health Services, Maasai Mara University, P. O. Box 861-20500, Narok, KENYA.
2Department of Pharmacy, University Health Services, Maasai Mara University, P. O. Box 861-20500, Narok, KENYA.
3Department of Pharmacy, School of Medicine and Health Sciences, Kenya Methodist University, P.O. BOX 267-60200 Meru, KENYA.
4Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053-00625, Nairobi, KENYA.
5Department of Pharmacy, University Health Services, Maasai Mara University, P. O. Box 861-20500, Narok, KENYA.

ABSTRACT
Background: The use of conventional cancer medication is limited by cytotoxicity on normal cells, intolerability of the drugs used and emergence of aggressive tumors which do not respond to treatment. Herbal alternatives are now being touted to be of promising efficacy. Fagaropsis angolensis (FA) has wide ranging ethno medicinal uses in Kenya. However, the anticancer potential of this plant is yet to be fully explored. The present study aims to determine the antiproliferative activity of crude extracts of Fagaropsis angolensis (FA) against African monkey kidney (Vero, E6), throat cancer (Hep2) and colon cancer (CT 26-CL 25) cell lines. Methods: Water and methanol extracts of FA were qualitatively screened to determine their phytochemical composition. In vitro growth inhibition capacity of these extracts on African monkey kidney (Vero, E6), throat cancer (HeP2) and colon cancer (CT26-CL25) cell lines was then assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium assay and expressed as 50% inhibitory concentration (IC50). Doxorubicin (standard anticancer agent) was used for comparison. Results: On Vero cell lines, statistical differences (p<0.05) were noted between the IC50 values of Doxorubicin and the methanol root stem extract of FA (19.00±9.00μg/ml against 8.33±1.42μg/ml) as well as between Doxorubicin and methanol whole root extract of FA (19.00±9.00μg/ml against 5.25±0.35μg/ml). The effects of the extracts of FA on throat cancer cell lines were unremarkable. Conclusions: These findings suggest that the choice of solvent may have some effect on the IC50 values of the extracts on cancer cell lines. It may also be suggested that the methanol root stem and whole root extracts of FA may be sources of important lead molecules that may be useful in the treatment of colon cancer. Conclusion: These findings suggest that the methanol root stem and whole root extracts of FA may be sources of important lead molecules in cancer therapy.

Key words: Fagaropsis angolensis, Kenya, Cancer, Doxorubicin.

Correspondence:
Dr. Antony Letoyah Yiaile,
Department of Pharmacy, University Health Services, Maasai Mara University,
P. O. Box 861-20500, Narok, KENYA.
Tel: +254721278085
E-mail: antonyaile@gmail.com
DOI: 10.5530/pc.2018.2.15

INTRODUCTION
The field of cancer research has been one of the greatest beneficiaries of plant derived products. Camptothecin and its analogues from Taxus brevifolia1, vincristine, vinblastine from Catharanthus roseus 2 and paclitaxel from Taxus brevifolia3 are examples of plant derived drugs that have significantly improved cancer treatment outcomes.4,5,6 However, therapy induced toxicity on normal body cells, the emergence of aggressive and therapy resistant tumors, as well as low selectivity indices of some chemotherapeutic agents has limited the efficacy of current conventional therapy.4,5,6,7 Therefore, there is a need to search for new sources of bioactive compounds that may serve as starting material in the drug development process.

Fagaropsis angolensis is a tree that belongs to the Rutaceae family.8 In Kenya, the stem bark is used in ethno medical treatment of malaria while the root is chewed as an expectorant.9,10 The antiproliferative activity of this plant is yet to be reported. The present study was conducted to evaluate the antiproliferative activity of Fagaropsis angolensis on throat and colon cancer cell lines.

MATERIALS AND METHODS
Chemicals
Analytical grade reagents and chemicals were used in all the experiments. These were obtained from Sigma Aldrich (St. Louis, MO).

Collection and identification of plant material
Plant material of Fagaropsis angolensis was collected from Mount Kenya forest located at Irangi Forest station, Embu County (1750m above sea level) in Kenya. (Figure 1). The collected specimens were identified and authenticated by Mr. Patrick Chalo Mutiso, a botanist at the University of Nairobi, Chiromo Campus. A voucher specimen (ALY2015/01) was prepared in duplicate and deposited at the University of Nairobi Chiromo campus herbarium for future reference. Different parts of Fagaropsis angolensis are as shown on Figure 2.

Preparation of extracts
The method described by Okumu et al.11 was used for the extraction process with minor modifications. Briefly, plant materials were washed with running tap water to remove adhering soil particles. The material was then chopped to small pieces and air-dried under shade for 2 weeks. Dry plant materials were then powdered using an industrial grade grinder and weighed. Fifty (50) g of each part of the ground plant material were soaked separately with 200ml of methanol and water in a one-liter conical flask wrapped in aluminum foil. This setup was left to stand for 48 hours with constant mixing using a magnetic stirrer. The extracts were filtered and the methanol was evaporated under low pressure at a temperature not greater than 45°C using a rotary evaporator (Buchi, Switzerland AG). Aqueous extracts were dried by lyophilization. The resultant products were stored in well-closed containers awaiting use in biochemical assays.

Phytochemical screening of extracts
Qualitative methods12,13,14 were used to identify the phytoconstituents in the prepared extracts. The phytochemicals were graded on the basis of the intensity of colour produced from reactions observed in the test
tubes. Very high concentration was denoted as (+++), high concentration (++), moderate concentration (+) and nil (-) represented no observable reaction.

**Test for alkaloids (Dragendorff test)**

Approximately 50 mg of each of the extracts was dissolved in a sufficient amount of distilled water. Concentrated hydrochloric acid (HCl) was then added to each of the solutions and the mixture filtered. Two ml of this filtrate was collected in a test tube and 1 ml of dragendorff’s reagent was added along the inner wall of the test vessels.

**Test for anthraquinones (Borntraggers test)**

Five mg of each of the extracts was added with 10 ml of benzene and the resulting mixture shaken and filtered. To each filtrate, 5 ml of 10% ammonia solution was added and the mixture agitated.

**Test for cardiac glycosides (Keller-killiani test)**

Half a gram of each of the extracts was diluted with 5 ml of water. Two ml of glacial acetic acid was then added followed by 2 drops of Ferric chloride solution (FeCl₃). Thereafter, 1 ml of concentrated sulphuric acid (H₂SO₄) was added along the inner walls of the reaction vessels.

**Test for flavonoids (Alkaline reagent test)**

Five ml of the each of the test extract solutions were taken in a test vessel. Thereafter, 0.5ml of 2M hydrochloric acid (HCl) was added.

**Test for phenolics (ferric chloride test)**

Two mg of each of the extracts was dissolved with 1 ml of each of the extracts. Thereafter, a few drops of 10% aqueous ferric chloride (FeCl₃) solution was added to each test tube.

**Test for phytosterols (steroids) (Liebermann-Burchard’s test)**

Two mg of each of the extracts was dissolved in acetic anhydride and the mixture boiled then allowed to cool. A volume of 1 ml concentrated sulphuric acid was then added along the inner walls of the test vessels.

**Test for saponins (foam test)**

Five ml of the each of the test extract solutions were taken in a test vessel and vigorously agitated for a period of five (5) min.

**Test for tannins (ferric chloride test)**

Five % ferric chloride (FeCl₃) solution was added to 2 ml of each of the test extract solutions.

**Test for terpenoids (Salkowski test)**

Two mg of the extracts were shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added along the inner walls of the reaction vessels.

**Human cancer cell lines and culture conditions**

The method of Bibi et al.

was used with slight modifications. Briefly, Vero E6 (normal cell), CT 26-CL 25 (colon cancer), and Hep2 (throat cancer) cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were initially stored in liquid nitrogen and thawed in a water bath at 37°C at the time of experimenta

**Statistical analysis**

Data was expressed as a mean ± standard error of the mean (SEM) of two independent experiments. Analysis was done by determining IC₅₀ (the concentration required to inhibit 50% growth of the cells) using linear regression curves. The level of toxicity of the crude extracts was assigned in respect to the toxicity grading scale by Loomis and Hayes.

**Ethical considerations**

Approval was sought from the biosafety, animal use and ethics committee (BAUEC) of the Faculty of Veterinary Medicine, University of Nairobi (Ref J56/73786/2014). All the used cell lines were disposed in accordance with Kenya Medical Research Institute (KEMRI)-University of Nairobi (UoN) protocols.

**RESULTS**

**Extraction yield**

Different plant parts of Fagaropsis angolensis were extracted with water and methanol to yield ten (10) extracts (Table 1).
Table 1: Yield and phytochemical composition of extracts of different plant parts of *Fagaropsis angolensis*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield (%)</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Phenols</th>
<th>Saponins</th>
<th>Steroids</th>
<th>Tannins</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-W</td>
<td>13.73</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-ME</td>
<td>6.54</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LS-W</td>
<td>12.60</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LS-ME</td>
<td>6.58</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RS-W</td>
<td>7.81</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RS-ME</td>
<td>6.48</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>RB-W</td>
<td>9.93</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>RB-ME</td>
<td>7.16</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WR-W</td>
<td>10.37</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>WR-ME</td>
<td>8.42</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: L–W; aqueous leaf extract, L-ME; methanol leaf extract, LS-W; aqueous leaf stalk extract, LS-ME; methanol leaf stalk extract, RS-W; aqueous root stem extract, RS-ME; methanol root stem extract, RB-W; aqueous root bark extract, RB-ME; methanol root bark extract, WR-W; aqueous whole root extract, WR-ME; methanol whole root extract.

Table 2: Concentration of extracts responsible for 50% inhibition of the growth of various cell lines.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vero E6 cell line</th>
<th>Hep2 cell line</th>
<th>CT26 cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W</td>
<td>M</td>
<td>W</td>
</tr>
<tr>
<td>Leaf</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Leaf stalk</td>
<td>&gt;100</td>
<td>1.10±0.70</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Root stem</td>
<td>&gt;100</td>
<td>59.70±3.80</td>
<td>60.50±0.00</td>
</tr>
<tr>
<td>Root bark</td>
<td>&gt;100</td>
<td>71.80±5.50</td>
<td>60.25±2.75</td>
</tr>
<tr>
<td>Whole root</td>
<td>&gt;100</td>
<td>5.80±0.80</td>
<td>21.65±0.05</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>6.5±3.25</td>
<td>2.5±0.50</td>
<td>19.00±9.00</td>
</tr>
</tbody>
</table>

Phytochemical composition of crude extracts of *Fagaropsis angolensis*

Phytochemical screening of the extracts revealed various secondary metabolites as shown on Table 1.

Inhibition of cancer cell growth by different plant part extracts of *Fagaropsis angolensis*

Figures 3-7 represent the effects of incubating the various cell lines with crude extracts of *Fagaropsis angolensis* over a 24 h period. From the graphs, the crude extracts exerted a dose dependent decrease in the growth of the cell lines.

In vitro cytotoxicity of crude extracts of *Fagaropsis angolensis* on Vero cell lines

The concentration of the crude extracts effective in inhibiting 50% growth of Vero cells (IC$_{50}$) is as presented on Table 2. All aqueous extracts had IC$_{50}$ values >100 µg/ml. Similar IC$_{50}$ values were also reported for the methanol extracts of *F. angolensis* with the exception of the methanol root stem and whole root extracts, which exhibited IC$_{50}$ values of 1.10±0.70 µg/ml and 5.10±0.80 µg/ml respectively compared to 6.5±3.25 µg/ml produced by the reference drug, doxorubicin.

In vitro cytotoxicity of crude extracts of *Fagaropsis angolensis* on Hep2 cell lines

The concentration of the extracts effective in inhibiting 50% growth of Hep2 cell line (IC$_{50}$) is as presented on Table 2. The aqueous leaf and leaf stalk extracts of *F. angolensis* and the methanol leaf extract of *F. angolensis* had IC$_{50}$ values of >100 µg/ml. The methanol whole root extracts of *F. angolensis* produced an IC$_{50}$ value of 10.05±2.15 µg/ml while the methanol leaf stalk extract of *F. angolensis* showed the highest value of 89.2±3.80 µg/ml compared to 2.5±0.50 µg/ml of the reference drug, doxorubicin.

In vitro cytotoxicity of crude extracts of *Fagaropsis angolensis* on CT26 cell lines

The concentration of the crude extracts of *Fagaropsis angolensis* effective in inhibiting 50% growth of the CT26 cell line (IC$_{50}$) are as presented on Table 2. Aqueous leaf, leaf stalk, root and root stem extracts of the *F. angolensis* leaf as well as the methanol leaf stalk extract of *F. angolensis* had IC$_{50}$ values of >100 µg/ml. Other extracts had varied IC$_{50}$ values with the aqueous whole root extracts of *F. angolensis* having the highest value of 85.20±2.70 µg/ml while the methanol root stem extract of *F. angolensis* had an IC$_{50}$ value of 8.33±1.42 µg/ml as compared to 19.00±9.00 µg/ml of the reference drug, doxorubicin.

DISCUSSION

In the present study, water extracts of *Fagaropsis angolensis* had higher yields than methanol extracts. According to Okumu et al., differences in solvent polarity may influence extract yields. However, high extract yields may not always translate to higher biological activity of medicinal plant extracts because interfering substances in medicinal plant extracts may contribute to high extract yields. Qualitative phytochemical screening of medicinal plant extracts is important in establishing a relationship between the pharmacological effects and the traditional uses the plants are associated with.
In the present study, we identified alkaloids, flavonoids, glycosides, phenols, steroids, tannins and terpenoids as secondary metabolites. These have been linked to a raft of therapeutic properties including anticancer activity.\cite{20,21} Moreover, a large body of literature is available on the efficacy of flavonoids with regard to chemoprevention and chemotherapy.\cite{20,22-24} High cost, toxic nature and rapid development of resistance have become synonymous with conventional cancer treatment.\cite{25,26} As such, there is an overwhelming need to invest in research that aims to identify new lead compounds that may address these challenges. In a bid to investigate the efficacy of new strategies of cancer therapy, cancer cell lines have been derived from human cells.\cite{27} The first cultured cancer cell line was the HeLa which was derived from tumour cells obtained from Henrietta Lacks in 1951.\cite{28} Since then, this field of science has become a widely accepted methodology for high throughput screening of drugs in biomedical research.\cite{29} In a bid to shed more light on the antiproliferative activity of crude extracts of *Fagaropsis angolensis* on cancer cell lines, three cell lines were selected for study; the cancerous HeP2, CT26 cell lines and the non-cancerous Vero cell line which served as a control.
After incubating the crude extracts of *Fagaropsis angolensis* with the cell lines over a 48 h period, we identified a dose dependent decrease in the growth of both the cancerous and normal cell lines. This is in agreement with the findings of other workers.\(^30\) The rationale behind the use of Hep2 and CT26 was based on the fact that colon and throat cancers are among some of the common cancers that afflict populations in developing countries,\(^31\) yet very little literature is available on the treatment of these types of cancers.

In 1955, the United States National Cancer Institute (NCI) established guidelines that set up limits of efficacy of crude medicinal plant extracts against cancer cell lines. Based on this criteria, crude extracts were considered to have promising anticancer potential if the concentration of the extracts effective in inhibiting 50% growth of cancerous cells (IC\(_{50}\)) was less than 30µg/ml.\(^32,33\) Moreover, Loomis and Hayes\(^17\) developed a scale for evaluating cytotoxicity of crude plant extracts. The scale categorizes an IC\(_{50}\) of less than 1 as extremely toxic, an IC\(_{50}\) of greater than 1 but less than 50 as highly toxic and an IC\(_{50}\) value of greater than 50 but less than 500 as moderately toxic. Previous workers have reported antiproliferative activity of *Fagaropsis angolensis* at a single concentration of 10µg/ml against mouth epidermoid (KB) and diploid embryonic lung (MRC-5) cancer cell lines.\(^34\) However, since cancer has been established to be of multifactorial etiology, it is important to test anticancer efficacy of medicinal plants in several cell lines\(^35\) as well as using several dilutions of the test substance. To the best of our knowledge, this is the first study to report antiproliferative activity of this plant against HeP2 and CT26 cancer cell lines. On the basis of the NCI criteria, IC\(_{50}\) values of the crude extracts that were below the prescribed 30 µg/ml limit was observed for the Hep2 and CT26 cancer cell lines. This data appears interesting as it implies that the crude extracts of *Fagaropsis angolensis* appear to be more selective to cancerous cells than normal cells. However, methanol root stem and whole root extracts of *Fagaropsis angolensis* were found to be highly toxic to normal cells. This is in agreement with the findings of Karakas and others\(^36\) who reported the effects of *Bellis perennis*, *Convolvulus galacticus*, *Trifolium pannonicum* and *Lysimachia vulgaris* on hepato-cellular carcinoma human cell lines. However, according to Otang and others,\(^37\) there is a need for concern when IC\(_{50}\) values of crude medicinal extracts on normal cell lines are very low. We share the opinion that the methanol extracts of the whole root and root stem require further investigations to establish their safety profile.

**CONCLUSION**

This study suggests that the methanol root stem and whole root extracts of FA may have promising activity against colon cancer. However, further work on a wider range of cancer cell lines may be important in confirming the anticancer potential of *Fagaropsis angolensis*. Moreover, there...
is a need to isolate and identify the chemical compounds responsible for anticancer activity.

**ACKNOWLEDGEMENT**

The research was carried out under the financial support of the Maasai Mara University, Research and Scholarship Fund (MMU-RSF). The authors wish to acknowledge Mr. Gervason Muriasi for assisting with the data analysis.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**ABBREVIATIONS**

FA: Fagaropsis angolensis; E6: Vero cell lines; HeP2: Throat cancer cell lines; CT26: Colon cancer cell lines; MTT: 3-(4,5-dimethylthiazol-2-yl-2, 5 diphenyltetrazolium); IC50: Inhibitory concentration at 50%; HCl: Hydrochloric acid; FeCl3: Ferric chloride; H2SO4: Sulphuric acid; NaOH: Sodium hydroxide; ATCC: American Type Culture Collection; EMEM: Eagles Modified Essential Medium; FBS: Fetal Bovine Serum; DMSO: Dimethylsulfoxide; PS: Penicillin-Streptomycin; SEM: Standard Error of the Mean; BAUEC: Biosafety, Animal Use and Ethics Committee; KEMRI: Kenya Medical Research Institute; KB: Mouth Epidermoid cancer cell line; MRC-5: Lung cancer cell line; MRC-7: Hepatocellular carcinoma.

**REFERENCES**


**ABOUT AUTHORS**

Dr. Yiaile is currently the head of the Health Services Department of the Maasai Mara University, one of the institutions of higher learning in Kenya. His areas of scientific interest include ethnopharmacology, chemistry of natural products, in vitro pharmacology and toxicology and clinical toxicology. He is currently involved in research that is exploring the use of nanomedicine in the management of malaria.

**HIGHLIGHTS OF PAPER**

- There was a dose dependent decrease in the growth of both cancerous (HeP2, CT26-CL) and normal cell lines (Vero, E6) upon treatment with crude extracts of *Fagaropsis angolensis*.
- Methanolic root stem and whole root extracts of *Fagaropsis angolensis* were more selective in inhibiting cancer cell line growth (HeP2, CT26-CL) than normal cell lines (Vero, E6).