ABOUT JMPR

The Journal of Medicinal Plant Research is published weekly (one volume per year) by Academic Journals.

The Journal of Medicinal Plants Research (JMPR) is an open access journal that provides rapid publication (weekly) of articles in all areas of Medicinal Plants research, Ethnopharmacology, Fitoterapia, Phytomedicine etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMPR are peer reviewed. Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jmpr@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Medicinal Plant Research will only accept manuscripts submitted as e-mail attachments.

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author.
Editors

Prof. Akah Peter Achunike
Editor-in-chief
Department of Pharmacology & Toxicology
University of Nigeria, Nsukka
Nigeria

Associate Editors

Dr. Ugur Cakilcioglu
Elazig Directorate of National Education
Turkey.

Dr. Jianxin Chen
Information Center,
Beijing University of Chinese Medicine,
Beijing, China
100029,
China.

Dr. Hassan Sher
Department of Botany and Microbiology,
College of Science,
King Saud University, Riyadh
Kingdom of Saudi Arabia.

Dr. Jin Tao
Professor and Dong-Wu Scholar,
Department of Neurobiology,
Medical College of Soochow University,
199 Ren-Ai Road, Dushu Lake Campus,
Suzhou Industrial Park,
Suzhou 215123,
P.R. China.

Dr. Pongsak Rattanachaikunsopon
Department of Biological Science,
Faculty of Science,
Ubon Ratchathani University,
Ubon Ratchathani 34190,
Thailand.

Prof. Parveen Bansal
Department of Biochemistry
Postgraduate Institute of Medical Education and Research
Chandigarh
India.

Dr. Ravichandran Veerasamy
AIMST University
Faculty of Pharmacy, AIMST University, Semeling - 08100,
Kedah, Malaysia.

Dr. Sayeed Ahmad
Herbal Medicine Laboratory, Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi, 110062, India.

Dr. Cheng Tan
Department of Dermatology, first Affiliated Hospital of Nanjing University of Traditional Chinese Medicine.
155 Hanzhong Road, Nanjing, Jiangsu Province, China. 210029

Dr. Naseem Ahmad
Young Scientist (DST, FAST TRACK Scheme)
Plant Biotechnology Laboratory
Department of Botany
Aligarh Muslim University
Aligarh- 202 002,(UP)
India.

Dr. Isiaka A. Ogunwande
Dept. Of Chemistry,
Lagos State University, Ojo, Lagos,
Nigeria.
Editorial Board

Prof Hatil Hashim EL-Kamali  
Omdurman Islamic University, Botany Department, 
Sudan.

Prof. Dr. Muradiye Nacak  
Department of Pharmacology, Faculty of Medicine, 
Gaziantep University, 
Turkey.

Dr. Sadiq Azam  
Department of Biotechnology, 
Abdul Wali Khan University Mardan, 
Pakistan.

Kongyun Wu  
Department of Biology and Environment Engineering, 
Guiyang College, 
China.

Prof Swati Sen Mandi  
Division of plant Biology, 
Bose Institute 
India.

Dr. Ujjwal Kumar De  
Indian Veterinary Research Institute, 
Izatnagar, Bareilly, UP-243122 
Veterinary Medicine, 
India.

Dr. Arash Kheradmand  
Lorestan University, 
Iran.

Prof Dr Cemşit Karakurt  
Pediatrics and Pediatric Cardiology 
Inonu University Faculty of Medicine, 
Turkey.

Samuel Adelani Babarinde  
Department of Crop and Environmental Protection, 
Ladoke Akintola University of Technology, 
Ogbomoso 
Nigeria.

Dr. Wafaa Ibrahim Rasheed  
Professor of Medical Biochemistry National Research Center 
Cairo 
Egypt.
ARTICLES

Research Articles

Polyphenol derivatives from bioactive butanol phase of the Tunisian narrow-leaved asphodel (Asphodelus tenuifolius Cav., Asphodelaceae) 576
Fathi Farag Mughrabi, Harita Hashim, Mahmood A. A, Suzy S. M, M. O. Oyeyemi and Ajani O. S.

Cytotoxic and cytogenetic effects of Convolvulus arvensis extracts on rhabdomyosarcoma (RD) tumor cell line in vitro 588
Asaad Abdulwahed Bader AL-Asady, Dhamia Kasem Suker and Kawthar Kalaf Hassan

Medicinal plant parts and practices used by communities around the Miombo woodlands of Urumwa, Tanzania 599
Suzana Augustino, John B. Hall, Fortunatus B. S. Makonda and Romanus C. Ishengoma
Full Length Research Paper

Effect of six environmental variables on five Bupleurum species distribution in Guandi, Wutai, Xiaowutai and Dongling mountains


College of Life Sciences, Beijing Normal University, Beijing 100875 P. R. China.

Received 2 April, 2011; Accepted 9 May, 2011

Effects of environmental factors on the spatial distribution of five Bupleurum genera were investigated. Eighty seven samples in total in four mountains were collected; 158, 116, 130 and 128 plant species were recorded in Guandi, Wutai, Xiao Wutai and Dongling mountains, respectively. This study has been focused on five particular species (Bupleurum bicaule Helm, Bupleurum scorzonerifolium Wildenow, Bupleurum sibiricum Vest, Bupleurum smithii H.Wolff and Bupleurum chinense DC) not only because of their medicinal properties, but also because of the threat they are subject to. Relations between environmental variables and their influence on these species distribution were analyzed using CANOCO software correlation statistical analysis. The distribution of these five species was found to be differently influenced by environmental variables. However altitude and vegetation type appeared to be the variables which influenced the most Bupleurum distribution in the four surveyed mountains. Preservation of these areas from human disturbance or nevertheless activity controlled by the establishment and strengthening of protected areas could be a solution for a sustainable management.

Key words: Bupleurum bicaule, Bupleurum scorzonerifolium, Bupleurum sibiricum, Bupleurum smithii, Bupleurum chinense, environmental variable, canonical correspondence analysis (CCA) multiple species response curves.

INTRODUCTION

Bupleurum genus of the Apiaceae family has from 250 to 455 genera and 3,300 to 3,700 species widely distributed in the temperate zone of both hemispheres, mainly in Eurasia and especially in Asia (Sofi et al., 2009; Menglan et al., 2005). Bupleurum genus is one of the fifteen commonly used herbs in the Traditional Chinese Medicine (TCM) (Subhuti, 1996). Day to day TCM is gradually recognized and applied in the world, making the increase demands for the TCM in the international market (China Patent Medicine Industry Report, 2010). Market development included differentiated demand for herbal product formulation, specially an increasing preference for products made of combinations of herbs over those based on single herbs (Blumenthal et al., 2006).

In 2005, the output value of TCM amounted to about €11 billion (1/4 of the overall output value in China's medical industry) and will rise to €18.8 billion in 2015. Customs figures show China exports 240,000 tons of medicines annually, of which 200,000 tons are raw herbs. The exported raw herbs accounted for 20% of the country's annual harvest. Many provinces such as Hebei, Guizhou, Yunnan, Sichuan, Shaanxi and Shanxi have

*Corresponding author. E-mail: Zhangjt@bnu.edu.cn. Tel: +86 10 58807647. Fax: +86 58807721.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
designated traditional medicine as a pillar industry (Helmut Kaiser Consultancy Studies, 2009).

Nowadays, because of their potential bio-active components as medicinal plant, exploitation of *Bupleurum* species for herbal purposes has become a new source of income for some Chinese farmers, but also source of species threat among which excessive picking and digging, deforestation, excessive herding and reclamation of grassland, tourism, urbanization, etc (www.biodiv.gov.cn). The herb material supply is cultivated or wild; this latter case is an issue which could lead from the shortage to the threat of defunctness of this genius which goes with seasonal variation. Indeed, in many surveyed places in Shanxi, Hebei provinces of China, where *Bupleurum* spp. were occurring few years ago, with peasants’ farms extension and grazing, some of these species are no more present nowadays. This study besides promoting conservation and diversification of these resources, also gives a sounding alarm on the management of these areas.

The present survey investigated the correlations between the distribution of five *Bupleurum* spp. and six environmental variables over four geographical environments in Guandi, Wutai, Xiao Wutai and Dongling mountains in China. The conclusion of this study gave an idea how these species responded to these environmental variables; and also could lead to some insights on how to plan environmental assessment and impact analysis which could contribute to the conservancy process of these important species.

**METHODOLOGY**

**Study area**

Guandi mountain is located in the West of Shanxi province of China, with the highest peak of 2831 m above the sea level (m a.s.l). Its geographical position is N37°20' to 38°20' and E110°18 to 111°18', and is part of Luliang mountain range (Gao and Zhang, 2006). The climate is a warm temperate semi-humid continental monsoon; the average annual temperature varies from 3 to 4°C. The annual frost-free period is about 100 days and the mean annual precipitation is about 838.8 mm (Zhang and Yang, 2008). Several soil types are encountered there, such as mountain cinnamon soil, brown forest soil and mountain meadow soil (Zhang and Meng, 2007); with the temperate coniferous forest (1650 to 1800 m), the broadleaf forest (1650 to 2150 m), the cold temperate coniferous forest (1800 to 2400 m) and the sub-alpine shrub meadow (2550 to 2831 m) (Yuan et al., 2004).

Wutai mountain located in the North side of Shanxi province of China between 38°30' to 39°15'N and 112°50' to 113°50'E, belongs to Taihang ranges and is one of the four Buddhist shrines of China (Ru and Feng, 2000). Wutai mountain area is a warm temperate semi-humid monsoon climate. The soil types from the foothills to the summit are: cinnamon soil, brown forest soil, and sub-alpine meadow soil. Wutai mountains have some typical characteristics of warm temperate deciduous broad-leave forest (Ru and Feng, 2000). Xiao Wutai mountain is located in Zhangjiakou, Hebei province of China. Its geographical coordinates are longitude 114°47' to 115°30' and latitude 39°50' to 40°07' with a top peak altitude of 2882 m a.s.l. Xiao Wutai mountain has a temperate continental monsoon climate with an average temperature of 6.4°C and annual rainfall varying from 400 to 700 mm (Liu et al., 2004). Xiao Wutai mountain area soils are cinnamon, mountain brown and sub-alpine meadow soils. Vegetation are (1) sub-alpine meadow, (2) subalpine shrub zone, (3) coniferous forest, (4) broad-leave forest zone, (5) broadleaf forest, (6) secondary shrub zone, and (7) fruit farm belt (Liu et al., 2004).

Dongling mountain (2303 m) is a mixed woodland, Beijing Forest Ecosystem Research Station of the Chinese Academy of Sciences, 117 km Western of Mentougou district of Beijing, its geographical position is 39°48’N to 40°02’N, 115°24’ to 115°36’E and located at an average altitude of 1075 m a.s.l (Liu et al., 2010; Xu and Zhang, 2008; Guo et al., 2004). The area is characterized by a temperate semi-humid monsoon climate with an annual temperature of 6.3°C. The annual mean precipitation is about 612 mm. The dominant soil type is cinnamon soil, with organic matter content of 8.8%, pH value of 6.34 and total nitrogen content of 0.45%. The main vegetation type is deciduous broadleaves forest of warm temperate zone (Liu et al., 2010).

**Data collection**

Data were collected from June to August, 2010. Along the altitudinal gradient between 1755 and 2714 m a.s.l, 1645 to 2502 m a.s.l, 1378 to 2194 m a.s.l and 1133 to 2303 m a.s.l, respectively for Guandi, Wutai, Xiao Wutai and Dongling mountains. Four types of sampling points were set up, and at least 5 quadrats around each sampling point were established randomly. Species data were recorded in each quadrat. The quadrats sizes were 10 m × 10 m for broadleaves, coniferous forest and shrubs; and 1 m × 1 m small quadrats were used to record herbs. The coverage of trees, shrubs and herbs were evaluated in each quadrat. Altitude, slope and slope aspect for each quadrat were also recorded. Altitude for each quadrat was measured by using an altimeter, the slope and slope aspect measured by using a compass meter (Zhang 2004; Zhang et al., 2006). Soil samples at a depth of 20 cm, in three random places were collected and mixed inside each quadrat for soil type and pH determination.

**Data analysis methods**

To appreciate the relations between *Bupleurum* spp. with environmental variables in each of the surveyed sites, Generalized Linear Model Multiples Species Response Curve have been used with a quadratic degree, Poisson distribution model, with a maximum value of binomial total value without transformation analysis of canonical correspondence analysis of CANOCO correlation statistical analysis program (Ter Braak and Smilauer, 2002). The pH of the soil has been measured in the supernatant suspension of an 1:5 soil:liquid (v/v) mixture according to the methods reference SA 06-ISO 10390 (1994) and Kissel and Vandrell (2004). The rope test has been used to figure out the type of collected soil (Dolezal, 2002).

**RESULTS**

**Relation Bupleurum spp. and altitude**

In Guandi mountain, *B. bicaule* occurred in the whole surveyed range area with a peak at around 2300 m a.s.l (Figure 1a). In Wutai mountain, *B. bicaule* showed a peak between 2000 and 2200 m a.s.l. *Bupleurum scorzonerifolium* occurred only between 1600 and 1700 m a.s.l with a peak approximately at 1650 m a.s.l (Figure 2a).
In Xiao Wutai mountain, *Bupleurum sibiricum* response curve showed a peak around 1600 m a.s.l. *Bupleurum smithii* response curve showed a narrow occurrence space, between approximately 2100 and 2300 m a.s.l with a peak at 2200 m a.s.l. (Figure 1c). In Dongling mountain, *B. sibiricum* occurred between 1800 and 2100 m a.s.l with a peak at 1900 m a.s.l. *B. smithii* occurred in the interval 1900 to 2400 m a.s.l with a peak at 2200 m a.s.l. *Bupleurum chinense* showed a peak at 1400 to 1600 m a.s.l. (Figure 1d).

The analysis of variance (ANOVA) revealed, four of the five *Bupleurum* spp. are correlated to altitude except *Bupleurum bicaule*. Stratification in distribution of *Bupleurum* spp. could be linked to the presence of different microclimates characteristic of each of the species, but the presence of *B. bicaule* in the whole range of study showed its independence to this factor (Table 1).

**Relation Bupleurum spp. and slope aspect**

In Guandi mountain, *B. bicaule* optimum response was between 20 and 40° with a peak at 30° (Figure 2a). In Wutai mountain, *B. bicaule* response increased the slope aspect without a peak (Figure 2b). *B. scorzonerifolium* curve shows an optimum between 0 and 20° and decreased progressively the slope aspect (Figure 2c). In Xiao Wutai mountain, *B. sibiricum* optimum response was between 30 and 50° with a peak at 40°. *B. smithii*. Occurred in nil and above 80° slope aspect (Figure 2d). In Dongling mountain, *B. sibiricum* occurred above 80°
Figure 2. Response curves of *Bupleurum bicaule* (black) and *Bupleurum scorzonerifolium* (red) *Bupleurum sibiricum* (blue), *Bupleurum smithii* (purple) and *Bupleurum chinense* (green) on the variable slope aspect in Guandi (a), Wutai (b, c), Xiao Wutai (d) and Dongling (e) Mountains.
slope aspect values. *B. smithii* occurred in slope aspect less than 20°. *B. chinense* occurred in the whole gradient with a peak between 40 and 70° slope aspect values (Figure 2e). Table 2 shows that the slope aspect was not a major factor in the distribution of *Bupleurum* spp. Observed difference could be linked to the variation of topography.

**Relation *Bupleurum* spp. and pH**

In Guandi mountain, *B. bicaule* response showed an optimum from pH 5.5 to 6.0 (Figure 3a). In Wutai mountain, *B. bicaule* response showed an optimum between pH 5.4 and 5.8. *B. scorzonerifolium* response peak was between pH 6.8 and 7 (Figure 3b). In Xiao Wutai mountain, *B. sibiricum* response curve increased with pH increase, this showed its preference for slightly to very slightly acid soil. *B. smithii* occurred in pH 5 to 5.4 with an optimum at 5.2 and showed its orientation for strongly acid soil (Figure 3c). In Dongling mountain, *B. chinense* occurred progressively with pH increase and showed its preference for very slightly acid, through neutral to very slightly alkaline soil (Figure 3b). *B. sibiricum* occurred between pH 4 and 5.5 with a peak at 4.75 and revealed its orientation for strongly acid soil (Figure 3b). *B. smithii* occurred between pH 5.5 and 6.25 with a peak at 5.75 and showed its preference for medium acid soil (Figure 3d).

The ANOVA revealed that *Bupleurum* spp. distribution is not correlated to pH. Soil composition could significantly make vary soil pH. A high or low concentration of OH ions could influence pH, leading to the observed differences (Table 3).

**Relation *Bupleurum* spp. and slope**

In Guandi mountain, *B. bicaule* slope occurrence range was between 0 and 40° with an optimum at approximately 20° (Figure 4a). In Wutai mountain, *B. bicaule* optimum response was at 30° while *B. scorzonerifolium* curve show no optimum, but a decrement until a lower point between 20 and 25° and an increment with the slope increased (Figure 4b). In Xiao Wutai mountain, *B. sibiricum* occurrence range was between 5 and 25° with an optimum at 15°. *B. smithii* lay along the entire horizontal gradient, but its response was high in slope values beyond 25° (Figure 4a). In Dongling mountain, *B. sibiricum* occurred in a narrow range from 25 to 35° with a peak at 30°. *B. smithii* occurred between 10 and 25° with a peak at 15°. *B. chinense* occurred between 0 and 10° and beyond 40° slope (Figure 4b).

The ANOVA showed that among the four surveyed sites, slope was a major factor in the *Bupleurum* spp. distribution in Dongling mountain. Indeed, different values of slope led to different exposure position to sunlight. Difference observed could be linked to the topography Variations (Table 4).

**Relation *Bupleurum* spp. and soil type**

Two types of soil have been recorded in the surveyed areas. Type 1 was the loamy soil and type 2 was the sandy soil (1 and 2 along the graph horizontal axis, respectively).

In Guandi mountain, *B. bicaule* response was a straight declining line from left to right, showing that *B. bicaule* occurred preferably in loamy soil (Figure 5a). In Wutai mountain, *B. bicaule* response to soil type was similar to the one in Guandi mountain, while *B. scorzonerifolium* response increased along the horizontal axis from left to right and show its preference for soil type 2, that is, sandy soil (Figure 5b). In Xiao Wutai mountain, *B. sibiricum* response was an increasing arc of circle from left to right, and showed its preference for sandy soil. *B. smithii* response was a decreasing arc of circle from left to right and showed its orientation for loamy soil (Figure 5c). In Dongling mountain, *B. chinense* response showed occurrence in both types of soil but with a preference for sandy soil. *B. sibiricum* and *B. smithii* occurred only in loamy soil, that is, type 1 (Figure 5d). The ANOVA showed that *B. sibiricum* distribution correlated with the soil type. Observed differences for the other species could be linked particularly to the ability of their adaptation ability (Table 5).

**Relation *Bupleurum* spp. and vegetation type**

Four types of vegetation have been surveyed in Xiao Wutai and Dongling mountains: type 1 is coniferous forest; type 2 is broadleaves forest; type 3 is shrubs land; and type 4 is grass land (1, 2, 3 and 4 along the graph horizontal axis, respectively).

In Guandi mountain, four types of vegetation have been surveyed: type 1 was coniferous forest; type 2 was broadleaves forest; type 3 was shrubs land; and type 4 was grass land (1, 2, 3 and 4 along the graph horizontal axis, respectively). In Guandi mountain, *B. bicaule* occurred preferably in vegetation types 1 and 4, that is, coniferous forests and grass land (Figure 6a). In Wutai mountain, *B. bicaule* was able to grow in the 4 types of environment, but preferably in vegetation type 4, that is, grass land. *B. scorzonerifolium* occurred only in vegetation type 2 (Figure 6b). In Xiao Wutai mountain, *B. sibiricum* response was optimum in the transitional zone between broadleaves forest and shrubs land, while *B. smithii* occurred preferably in grass land (Figure 6c). In Dongling mountain, *B. chinense* was able to occur in the whole range of the surveyed vegetation types, but the curve showed its orientation for broadleaves forest (Figure 6d). *B. sibiricum* and *B. smithii* occurred only and preferably in grass land (Figure 6d).

The ANOVA showed all the five species are subject to
Figure 3. Response curves of *Bupleurum bicaule* (black) and *Bupleurum scorzonerifolium* (red) *Bupleurum sibiricum* (blue), *Bupleurum smithii* (purple) and *Bupleurum chinense* (green) on the variable pH in Guandi (a), Wutai (b), Xiao Wutai (c) and Dongling (d) Mountains.
Figure 4. Response curves of Bupleurum bicaule (black) and Bupleurum scorzonerifolium (red) Bupleurum sibiricum (blue), Bupleurum smithii (purple) and Bupleurum chinense (green) on the variable slope in Guandi (a), Wutai (b), Xiao Wutai (c) and Dongling (d) Mountains.
Figure 5. Response curves of Bupleurum bicaule (black) and Bupleurum scorzonerifolium (red) Bupleurum sibiricum (blue), Bupleurum smithii (purple) and Bupleurum chinense (green) on the variable soil type in Guandi (a), Wutai (b), Xiao Wutai (c) and Dongling (d) Mountains.

DISCUSSION

B. smithii occurred in higher altitude compared to B. sibiricum and this latter occurs in higher one than B. chinense. In a global way, B. chinense occurred between 1400 and 1600 m a.s.l in accordance with Menglan and Watson (2005) who found that this species occurrence was between 1000 and 1700 m a.s.l in the studied geographical area. But our survey showed that B. chinense occurred in broadleaves forest and shrubs land contrary to Menglan et al. (2005) who found its occurrence in grass land. We have also found that in Dongling Mountain, B. chinense occurred in aspect 40 to 70°, in very slightly acid to neutral environment and in low slope 0 to 10°. B. smithii occurred between 2100 and 2300 m a.s.l in accordance with Menglan et al. (2005) who found its occurrence between 1400 and 3700 m a.s.l. This study also found that in Xiao Wutai Mountain, B. smithii occurred in aspect beyond 80°, in strongly and medium acid environment, in slope above 25°, in sandy soil, in coniferous forest and grass land. The previous results are not totally in accordance with the one in Dongling Mountain where B. smithii occurs in aspect less than 20, in medium acid environment, in slope between 10 and 25°, in loamy soil and in grassland.

B. sibiricum occurred between 1500 and 2100 m a.s.l in accordance with Danren and Li (1974) who found its occurrence between 1500 and 2000 m a.s.l. In Xiao Wutai Mountain, B. sibiricum occurred in altitude between 1500 and 1700 m a.s.l, in aspect 30 to 50°, in slightly acid environment, in slope 5 to 25°, in loamy soil and in shrubs land. Except occurrence in loamy soil common to the both sites, in Dongling mountain, B. sibiricum occurred in altitude between 1800 and 2100 ma.s.l, in aspect above 80°, in strongly acid environment...
Figure 6. Response curves of Bupleurum bicaule (black) and Bupleurum scorzonerifolium (red) Bupleurum sibiricum (blue), Bupleurum smithii (purple) and Bupleurum chinense (green) on the variable vegetation type in Guandi (a), Wutai (b), Xiao Wutai (c) and Dongling (d) Mountains.
in slope 25 to 35° and in grass land. Furthermore, vegetation and slope were the only variable to influence in some extent the five recorded *Bupleurum* spp.

**Conclusion**

The development, structure and function of an organism depend on the interaction of that organism with its environment. Differences in response could be explained by other environmental variables non-studied in this survey like soil composition, insolation, annual precipitation...
precipitation etc. For sustainable management, it would be difficult or impossible to control environmental factors; in this study, vegetation has emerged as a determining factor in the distribution of species. One of the most feasible solutions could be the preservation of these areas from human activity or nevertheless activity controlled by the establishment and strengthening of protected areas. Public awareness is also vitally important.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Foundation of China (No. 30870399). The authors thank Prof. Liu Quanru at the College of Life Sciences of the Beijing Normal University for his precious support in the certification of the collected species and also, Zhao Mingfei from the College of Life Sciences of Beijing Normal University, who participated actively in the field surveys.

Table 5. ANOVA summarized results on the variable soil type in the four sites.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Guandi Mountain</th>
<th>Wutai Mountain</th>
<th>Xiao Wutai Mountain</th>
<th>Dongling Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. bicaule</td>
<td>B. bicaule</td>
<td>B. scorzonerifolium</td>
<td>B. sibiricum</td>
</tr>
<tr>
<td>Soil Type</td>
<td>0.46b</td>
<td>0.44b</td>
<td>0.30b</td>
<td>0.04b</td>
</tr>
<tr>
<td></td>
<td>0.06b</td>
<td>0.05a</td>
<td>0.03b</td>
<td>0.15b</td>
</tr>
<tr>
<td>Threshold P</td>
<td>≤ 0.05</td>
<td>≥ 0.05</td>
<td>≥ 0.05</td>
<td>≤ 0.05</td>
</tr>
</tbody>
</table>

Table 6. ANOVA summarized results on the variable vegetation type in the four sites.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Guandi Mountain</th>
<th>Wutai Mountain</th>
<th>Xiao Wutai Mountain</th>
<th>Dongling Mountain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. bicaule</td>
<td>B. bicaule</td>
<td>B. scorzonerifolium</td>
<td>B. sibiricum</td>
</tr>
<tr>
<td>Vegetation type</td>
<td>0.16b</td>
<td>0.04a</td>
<td>10^6a</td>
<td>0.08b</td>
</tr>
<tr>
<td></td>
<td>4×10^5a</td>
<td>0.02b</td>
<td>10^5a</td>
<td>9.5×10^5a</td>
</tr>
<tr>
<td>Threshold P</td>
<td>≤ 0.05</td>
<td>≥ 0.05</td>
<td>≥ 0.05</td>
<td>≤ 0.05</td>
</tr>
</tbody>
</table>

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


This study was supported by the National Natural Foundation of China (No. 30870399).


Cytotoxic and cytogenetic effects of *Convolvulus arvensis* extracts on rhabdomyosarcoma (RD) tumor cell line *in vitro*

Asaad Abdulwahed Bader AL-Asady¹*, Dhamia Kasem Suker² and Kawthar Kalaf Hassan³

¹School of Medicine, Faculty of Medicine, Duhok University, Iraq.
²Department of Biology, College of Science, Basrah University, Iraq.
³College of Medicine, Basrah University, Iraq.

Received 6 October, 2014; Accepted 4 April, 2014

The present study was designed to investigate the cytotoxicity of (aqueous and methanol) crude extracts of the leaves, stems and roots extracts as well as proteoglycan and glycoside fraction I (FI) of *Convolvulus arvensis* against human Rhabdomyosarcoma (RD) tumor cell line *in vitro*. The effect of glycoside FI fraction on mitotic index (MI) of RD cell line was investigated as well. The optical density (OD) of cell growth was measured by Elisa reader at 492 nm using tetrazolium bromide (MTT). Aqueous and methanol leaves extracts and glycoside FI had more cytotoxic effects at 10 mg/ml after 24 h. After 48 h, proteoglycan and glycoside FI at 10 mg/ml revealed very high cytotoxic activity compared with other concentrations. After 72 h, glycoside FI at 10 mg/ml had more cytotoxic inhibition compared with other extracts. Glycoside FI had cytotoxicity concentration 50% (CC 50%) 1.775, 0.870 and 0.706 mg/ml after 24, 48, and 72 h, respectively. The root aqueous extract had less cytotoxic effect after 72 h than other extracts; the CC 50% was 7.437 mg/ml. Cytotoxicity of root aqueous extract was more pronounced at higher concentration of 10 mg/ml. The effect of glycoside FI on MI of RD tumor cell line was concentration dependant.

**Key words:** Cytotoxicity, cytogenetics, *Convolvulus arvensis*, rhabdomyosarcoma (RD) tumor cell line, mitotic index (MI), tetrazolium bromide (MTT).

INTRODUCTION

Herbal medicine has a vital role in the prevention and treatment of cancer. A great deal of pharmaceutical research output in advanced countries has considerably improved the quality of the herbal medicines used in treatment of cancer (World Health Organization (WHO), 2002). The Convolulaceae family includes a large number of important plants which have many chemical compounds that are used for treating many diseases (Jacobs and NRCS, 2007).

*Convolvolus* species are widely distributed all over the world in different localities; some of them have medicinal activity (Abdel-Raheim et al., 2011), such as anticancer, anti-ulcerogenic and antidiarrhoeal. Some species also have a broad activity against some bacteria and fungi (Dhingra and Valecha, 2007). *Convolvulus arvensis* is an example of a medicinal plant with high therapeutic activities. *C. arvensis* is evaluated as a potential new source of antioxidant activity (Mohammed et al., 2011).

*Corresponding author. E-mail: Alasady.asaad@uod.ac. Tel: 009647504711189.*

Author(s) agree that this article remain permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](http://creativecommons.org/licenses/by/4.0/)
The antioxidant activity of *C. arvensis* extracts is mainly due to phenolic contents such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Awaad and Jaber, 2010; Thakral et al., 2010). *C. arvensis* may be a promising source of anticancer agents (Thane et al., 2000). Different extracts of *C. arvensis* affect tumor angiogenesis and immune cell function that stimulate immune cells (Kidd, 2000). Angiogenesis inhibitors derived from natural sources include flavonoids, sulphated carbohydrates and triterpenoids (Paper, 1998). Bird weed extract which contains angiogenesis-suppressing proteoglycan molecules (PGMs) regulates the production of a potent angiogenesis inhibitor, interleukin 12. All the previous researches revealed that no study has been carried out on cytotoxicity and cytogenetic effects of different extracts of *C. arvensis* on rhabdomyosarcoma (RD) tumor cell line. Therefore the present study was conducted to evaluate the cytotoxic effects of eight of extracts of *C. arvensis* and cytogenetic effect of glycoside FI leaves fraction of *C. arvensis* on this cell line.

**MATERIALS AND METHODS**

**Aqueous extracts**

An aqueous extract from leaves, stems or roots of *C. arvensis* was prepared according to Harborne (1984). Fifty grams of the dried ground leaves, stems or roots were macerated with 200 ml water over night, at 45°C. After 24 h, the extractive solution was filtered in a double layer of gauze then through filter paper (Whitman No.1). The pooled extract was evaporated to dryness at 45°C under reduced pressure in a rotary evaporator (Orem Scientific Ltd, Swiss). The yield of crude extract was weighted and kept at -20°C until use. Half gram of resultant extract was dissolved into 10 ml phosphate buffer saline (PBS). The suspension was filtered and sterilized by using two sterile Millipore filter papers, 0.45 and 0.22 μm and was kept in deep freeze at -20°C as a stock solution until use.

**Methanol crude extracts of *C. arvensis***

Leaves, stems and root methanol extracts were prepared according to Lin et al. (2010). Fifty gram of each part was refluxed with 250 ml of absolute methanol at 60°C for 4 h. The supernatant was separated from the solid residue using Whitman No.1 filter paper. The extraction was repeated twice. The extracts were combined and evaporated at 60°C under reduced pressure. After drying, 0.5 g of the resultant extract was dissolved in 10 ml PBS. The suspension was filtered and sterilized using both 0.45 and 0.22 μm sterile Millipore filter paper and was kept in deep freeze at -20°C as a stock solution until use.

**Extraction and fractionation of glycosides from leaves of *C. arvensis***

Glycosides were separated and fractionated according to Menemen et al. (2002). The resultant extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator (Orem Scientific Ltd., Swiss). To a fractionation of glycoside, the dried glycoside extract was dissolved in 80% methanol and was run by thin layer chromatography (TLC) using silica gel in n-butanolic acid:water (BAW), 5:1:4 as eluent. The glycoside spots were examined and their position and color reactions recorded and finally the rate of flow (Rf) values were recorded. To obtain a large amount of fractions, we used the protocol of Menemen et al. (2002). Half gram of the FI was dissolved in 10 ml of 1% dimethyl sulfoxide (DMSO) and the suspension filtered and sterilized using 0.45 μm then a 0.22 μm sterile Millipore filter paper kept in deep freeze -20°C as a stock solution to be used later.

**Extraction of proteoglycan molecules (PGM) from leaves of *C. arvensis***

The dried powder of *C. arvensis* leaves was mixed in dry weight (DW) at a concentration of 0.16 g/ml to prepare the proteoglycan as described previously (Meng et al., 2002). The resultant product was lyophilized to produce the extract powder which is referred to as PGM. 0.5 gm of resultant extract was dissolved in 10 ml PBS and the suspension filtered and sterilized using 0.45 μm then a 0.22 μm sterile Millipore filter paper, and finally kept in deep freeze -20°C as a stock solution until use.

**Chemical tests**

Alkaloids in plant extracts were determined using Wagner's and Dragendorff tests (Harborne, 1984), whereas tannins were determined using ferric chloride and lead acetate solutions (Harborne, 1984). Flavonoids was tested according to AL-Shahaat (1986). Liebermann-Burchard test was used for triterpenoids. Peptides and free amino groups tests were used for peptides, primary or secondary amino groups (Harborne, 1984). Molish reagent test was used for carbohydrate (Hawk et al., 1954). The presence of glycosides was detected according to AL-Shahaat (1986). Saponins were identified according to Harborne (1984). Biuret test was used to detect protein (Saadalla, 1980).

**Cell lines**

Rhabdomyosarcoma (RD) was provided by Iraqi Center for Cancer and Medical Genetic Research/Baghdad (ICCMGR). Passage number 45 was used and the cells were cultivated in minimum essential medium (MEM) with L-Glutamine and HEPES (Sigma, USA) which was supplemented with 10% of fetal calf serum and penicillin/streptomycin. The following formula was followed to calculate viability of the cell lines using 1% Trypan blue stain (Fine Chemical, Sweden Pharmec): \( C = N \times D \times 10^5 \), where \( C \) is the number of viable cells per milliliter, \( N \) is the number of viable cells counted, and \( D \) is the dilution factor (\( D = 10 \)) (Fresheney, 1994).

**Effect of aqueous and methanol crude extracts of leaves, stems, root, proteoglycan and glycoside (FI) extracts of *C. arvensis* on growth of RD tumor cell line**

About 200 μl of RD cells passage (45) were suspended (55,000 cells/ml) in growth medium. These cells were seeded into each well of a sterile 96-wells micro-titration plate. The plates were sealed with a self-adhesive film, lids were placed on and incubated at 37°C in 5% CO2 incubator. When the cells are in exponential growth (approximately 70 to 80% confluent monolayer) after 72 h, the medium was removed and serial dilutions of each aqueous, methanol crude extracts of *C. arvensis* in free serum MEM medium were added to the wells. The same was applied for glycoside (FI) and proteoglycan (PGM) separately. The dilutions were 10, 5, 2.5, 1.250, 0.5, 0.313, 0.156 and 0.078 mg/ml, respectively. Three replicates were used for each concentration of each extract. Three wells were used for seeding cells in medium alone, another three
Feeding cells in medium with PBS, and three wells for each slide to calculate the M% removed. Slides of wells with plant extracts (Betancur et al., 1999). The cytotoxic concentration 50% (CC50) for each extract was calculated from concentration-effect-curves after linear regression analysis (Hayslett and Patrick, 1981).

**Results**

The results of qualitative chemical analysis of *C. arvensis* extracts

The results of qualitative chemical analysis of *C. arvensis* (aqueous and methanol) crude extracts from (leaves, stems and roots), methanol glycosides (FI and FII) and aqueous proteoglycan from leaves with respect to the yield of extractions % are summarized in Tables 1 and 2.

**Thin layer chromatography (TLC) of methanol leaves extract of *C. arvensis***

The results of TLC for methanol leaves extract of *C. arvensis* showed the presence of two brown color spots; the color was developed by H2SO4 as shown in Figure 1. These two spots were different in the rate of flow (Rf). FI = 0.8 and FII = 0.187 (Table 3).

**Spectrophotometer analysis**

The result of fractions of glycosides from leaves of *C. arvensis* showed two peak of Fractions I and II at λ = 450 nm (Figure 2).

**Fourier transforms infra-red (FTIR) spectroscopy analysis of glycoside fractions**

Infrared spectrum for glycoside fractions FI and FII extracted from *C. arvensis* leaves are shown in Figures 3 and 4 and the results were illustrated in Table 4.

**Melting point**

The melting point of Fraction I was at 160°C, but the melting point of fraction II was not detected due to its viscous nature.

Cytogenetic effect of glycoside (FI) extract on RD tumor cell line

Cultures of RD tumor cell line (three replicates) were used for treatment with glycoside FI extract since it was a more effective extract to determine the mitotic index (MI). The concentrations of glycoside FI were 0.353, 0.176 and 0.088 μg/ml, which were selected according to the cytotoxicity tests (less than CC50%). Another set of three culture flasks were used to maintain the media with 1% DMSO only as a negative control. Flasks were incubated at 37°C for 72 h (Modi et al., 1987). Culture in the flask was re-fed with pre-warmed fresh medium 6 h before adding colcemide solution to obtain a final concentration of 1 μg/ml and incubated at 37°C for half an hour. Slides were prepared according to Modi (1987) and stained by using 2% Giemsa stain (Merck, USA) for 2.5 min and rapidly washed with Sorenson's buffer and then left to dry at room temperature. Microscopical examination under 40× objective lens was followed to detect the MI. One thousand cells were examined in each slide to calculate the MI. The MI% was determined as a ratio of the mitotic cells in metaphase to the total cells.

\[
\text{MI} = \left( \frac{\text{No. of dividing cells}}{\text{No. of dividing cells} + \text{No. of non-dividing cells}} \right) \times 100
\]

(Kleinsmith, 2006).

**Statistical analysis**

The results were evaluated by the analysis of the variance (ANOVA), P-values at levels (P ≤ 0.01) were considered to be statistically significant and this calculation was carried out according to statistical package for social science (SPSS, version 19). The least significant difference (LSD) at the level less than 0.05 were used to determine the significant differences between levels of each factor (Steel and Torrie, 1980).

Figure 1. TLC of glycoside fractions developed by H2SO4.
Cytotoxic effects of (aqueous, methanol) crude extracts from leaves, stems and roots (glycoside FI and PGM) from leaves of *C. arvensis* on RD tumor cell lines *in vitro*

The results show that the effect of glycoside FI, proteoglycan extracts and all crude extracts (leaves, stems and roots) of *C. arvensis* on proliferation of RD tumor cell line was highly significant (*P* ≤ 0.001) in all periods of treatments. The interaction between the effects of extracts and their concentrations was highly significant (*P* ≤ 0.001) after all periods of treatments. The foregoing
foregoing results indicate that the toxicity of the leaves stems and roots extracts varied with different types of extracts and concentrations. The concentrations of leaves methanol, leaves aqueous extracts and glycoside F1 10 mg/ml after 24 h show significant effect on growth of RD tumor cell line (Table 5) as compared to the control group which shows complete confluent monolayer of cohesive malignant cells. The effect of each stems aqueous extract, proteoglycan and stem methanol extract at 10 mg/ml was less than that of leaves methanol, leaves aqueous extract and glycoside F1 at 24 h. Interaction between concentrations and extracts revealed that leaves methanol, leaves aqueous extracts and glycoside F1 extract had the same effect at 10 mg/ml after 24 h. After 48 h, Table 6 shows that the effect of proteoglycan and glycoside F1 against the proliferation of RD cells, especially at 10 mg/ml, was more than the effects of all others extracts.
Table 2. Qualitative chemical analysis for aqueous and methanol extracts of leaves, stems, roots, glycosides FI, FII and proteoglycan for leaves of *C. arvensis*.

<table>
<thead>
<tr>
<th>Compound group</th>
<th>Extract</th>
<th>Aqueous</th>
<th>Methanol</th>
<th>Proteoglycan</th>
<th>Glycosides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Stem</td>
<td>Root</td>
<td>Leaves</td>
<td>Stem</td>
</tr>
<tr>
<td>Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wagner's test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dragendorf test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyphenol (Tannins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcoholic KOH</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulfuric acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid (Liebermann-Burchard test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peptides free amino group</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate (Molish test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides (Benedict)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>After hydrolysis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein (Biuret test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polyphenol (Tannins)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ferric chloride</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = The extract contain the designated phytochemicals; - = The extract does not contain the designated phytochemicals.

Table 3. Thin layer chromatography for glycosides (Rf and color of spots).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rate of flow (Rf)</th>
<th>Color of spot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycosides</td>
<td>FI</td>
<td>0.8</td>
</tr>
<tr>
<td>Glycosides</td>
<td>FII</td>
<td>0.187</td>
</tr>
</tbody>
</table>
Table 4. The main functional groups and their frequencies in FTIR of fraction I and fraction II.

<table>
<thead>
<tr>
<th>Band frequency (cm⁻¹)</th>
<th>Group</th>
<th>Mode of vibration</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI</td>
<td>FII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3423 (br)</td>
<td>3411 (br)</td>
<td>-OH</td>
<td>Stretch</td>
</tr>
<tr>
<td>2927</td>
<td>2927</td>
<td>C-H</td>
<td>Stretch</td>
</tr>
<tr>
<td>1724</td>
<td>1724</td>
<td>C=O</td>
<td>Stretch</td>
</tr>
<tr>
<td>1602</td>
<td>1614</td>
<td>C=C</td>
<td>Stretch</td>
</tr>
<tr>
<td>1515</td>
<td>1573</td>
<td>C=C</td>
<td>stretch</td>
</tr>
<tr>
<td>1452</td>
<td>1423</td>
<td>C-H</td>
<td>Asymmetric bending</td>
</tr>
<tr>
<td>1384</td>
<td>-</td>
<td>-OH</td>
<td>Bend in plane</td>
</tr>
<tr>
<td>1272</td>
<td>1261</td>
<td>C-O-C</td>
<td>Asymmetric stretch</td>
</tr>
<tr>
<td>1070</td>
<td>1076</td>
<td>C-O-C</td>
<td>Symmetrical stretch</td>
</tr>
</tbody>
</table>

Table 5. Mean ± SE for the effect of different concentrations of (SM, SA, LM, RM, RA, LA, glycoside (FI) and proteoglycan extracts of C. arvensis on the proliferation of RD tumor cell line after 24 h treatments in vitro (observations of OD).

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentration mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Stem methanolic</td>
<td>0.739±0.001</td>
</tr>
<tr>
<td>Stem aqueous</td>
<td>0.739±0.004</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>0.739±0.021</td>
</tr>
<tr>
<td>Leaves methanolic</td>
<td>0.794±0.001</td>
</tr>
<tr>
<td>Root methanolic</td>
<td>0.794±0.001</td>
</tr>
<tr>
<td>Root aqueous</td>
<td>0.794±0.001</td>
</tr>
<tr>
<td>Leaves aqueous</td>
<td>0.765±0.005</td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.747±0.003</td>
</tr>
</tbody>
</table>

SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous, LA = leaves aqueous. LSD = least significant difference.

After 72 h, proteoglycan and glycoside FI possessed an activity from 0.156 to 10 mg/ml; their activity against the growth of RD cells increased by increasing their concentration. The results showed that glycoside FI was more effective extract against the proliferation of RD cells especially at 10 mg/ml where the value of OD was 0.105 ± 0.002 (Table 7).

The exposure times had a highly significant effect (P ≤ 0.001) on growth of RD tumor cell line treated with stems methanol extract, stems aqueous extract, proteoglycan and leaves aqueous extracts. Leaves and roots methanol extracts had less significant effect (P ≤ 0.01). Time was not effective significantly on growth of
Table 6. Mean ± SE for the effect of different concentrations of SM, SA, LM, RM, RA, LA, Glycoside FI and proteoglycan extracts of *C. arvensis* on the proliferation of RD tumor cell line after 48 h treatments *in vitro* (observations of OD).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration mg/ml</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.078</td>
<td>0.156</td>
<td>0.312</td>
<td>0.625</td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Stem methanolic</td>
<td>0.803±0.005</td>
<td>0.787±0.031</td>
<td>0.749±0.041</td>
<td>0.584±0.043</td>
<td>0.575±0.021</td>
<td>0.557±0.018</td>
<td>0.399±0.036</td>
<td>0.390±0.011</td>
</tr>
<tr>
<td>Stem aqueous</td>
<td>0.803±0.005</td>
<td>0.795±0.009</td>
<td>0.767±0.027</td>
<td>0.458±0.005</td>
<td>0.330±0.04</td>
<td>0.324±0.024</td>
<td>0.325±0.023</td>
<td>0.183±0.002</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>0.803±0.005</td>
<td>0.803±0.021</td>
<td>0.673±0.026</td>
<td>0.705±0.002</td>
<td>0.372±0.012</td>
<td>0.371±0.005</td>
<td>0.372±0.016</td>
<td>0.094±0.002</td>
</tr>
<tr>
<td>Leaves methanolic</td>
<td>0.798±0.041</td>
<td>0.789±0.02</td>
<td>0.787±0.06</td>
<td>0.741±0.031</td>
<td>0.640±0.009</td>
<td>0.187±0.005</td>
<td>0.172±0.001</td>
<td>0.155±0.002</td>
</tr>
<tr>
<td>Root methanolic</td>
<td>0.798±0.041</td>
<td>0.798±0.011</td>
<td>0.797±0.015</td>
<td>0.766±0.004</td>
<td>0.754±0.064</td>
<td>0.631±0.077</td>
<td>0.231±0.017</td>
<td>0.225±0.001</td>
</tr>
<tr>
<td>Root aqueous</td>
<td>0.798±0.041</td>
<td>0.788±0.031</td>
<td>0.787±0.039</td>
<td>0.776±0.036</td>
<td>0.770±0.017</td>
<td>0.766±0.002</td>
<td>0.764±0.007</td>
<td>0.761±0.018</td>
</tr>
<tr>
<td>Leaves aqueous</td>
<td>0.762±0.052</td>
<td>0.458±0.015</td>
<td>0.461±0.03</td>
<td>0.458±0.027</td>
<td>0.483±0.018</td>
<td>0.412±0.004</td>
<td>0.416±0.006</td>
<td>0.386±0.022</td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.731±0.002</td>
<td>0.746±0.003</td>
<td>0.705±0.002</td>
<td>0.221±0.001</td>
<td>0.209±0.002</td>
<td>0.149±0.0005</td>
<td>0.139±0.001</td>
<td>0.121±0.001</td>
</tr>
</tbody>
</table>

**Effectors**

<table>
<thead>
<tr>
<th>LSD (0.05)</th>
<th>Extract</th>
<th>Concentration</th>
<th>Extracts and concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02336</td>
<td>0.02478</td>
<td>0.07009</td>
<td></td>
</tr>
</tbody>
</table>

SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

Table 7. Mean ± SE for the effect of different concentrations of (SM, SA, LM, RM, RA, LA, Glycoside (FI) and Proteoglycan) extracts of *C. arvensis* on the proliferation of RD tumor cell line after 72 h treatments *in vitro* (observations of OD).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration mg/ml</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.078</td>
<td>0.156</td>
<td>0.312</td>
<td>0.625</td>
<td>1.25</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Stem methanolic</td>
<td>0.756±0.028</td>
<td>0.756±0.00</td>
<td>0.436±0.015</td>
<td>0.317±0.023</td>
<td>0.225±0.018</td>
<td>0.201±0.014</td>
<td>0.216±0.014</td>
<td>0.216±0.004</td>
</tr>
<tr>
<td>Stem aqueous</td>
<td>0.756±0.028</td>
<td>0.756±0.014</td>
<td>0.351±0.014</td>
<td>0.338±0.007</td>
<td>0.273±0.035</td>
<td>0.237±0.015</td>
<td>0.223±0.011</td>
<td>0.207±0.012</td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>0.756±0.028</td>
<td>0.755±0.012</td>
<td>0.489±0.009</td>
<td>0.340±0.029</td>
<td>0.204±0.002</td>
<td>0.174±0.001</td>
<td>0.170±0.005</td>
<td>0.162±0.001</td>
</tr>
<tr>
<td>Leaves methanolic</td>
<td>0.794±0.002</td>
<td>0.783±0.008</td>
<td>0.779±0.005</td>
<td>0.679±0.002</td>
<td>0.521±0.002</td>
<td>0.164±0.001</td>
<td>0.144±0.002</td>
<td>0.141±0.001</td>
</tr>
<tr>
<td>Root methanolic</td>
<td>0.794±0.002</td>
<td>0.793±0.001</td>
<td>0.782±0.001</td>
<td>0.759±0.003</td>
<td>0.731±0.005</td>
<td>0.628±0.002</td>
<td>0.232±0.0005</td>
<td>0.158±0.001</td>
</tr>
<tr>
<td>Root aqueous</td>
<td>0.794±0.002</td>
<td>0.786±0.001</td>
<td>0.785±0.002</td>
<td>0.775±0.002</td>
<td>0.764±0.002</td>
<td>0.752±0.0005</td>
<td>0.697±0.002</td>
<td>0.571±0.002</td>
</tr>
<tr>
<td>Leaves aqueous</td>
<td>0.763±0.020</td>
<td>0.746±0.044</td>
<td>0.548±0.016</td>
<td>0.370±0.035</td>
<td>0.272±0.01</td>
<td>0.264±0.024</td>
<td>0.246±0.004</td>
<td>0.161±0.003</td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.718±0.001</td>
<td>0.716±0.001</td>
<td>0.681±0.001</td>
<td>0.208±0.002</td>
<td>0.199±0.001</td>
<td>0.143±0.001</td>
<td>0.136±0.002</td>
<td>0.117±0.001</td>
</tr>
</tbody>
</table>

**Effectors**

<table>
<thead>
<tr>
<th>LSD (0.05)</th>
<th>Extract</th>
<th>Concentration</th>
<th>Extracts and concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01215</td>
<td>0.01288</td>
<td>0.03644</td>
<td></td>
</tr>
</tbody>
</table>

SM = stem methanol, SA = stem aqueous, LM = leaves methanolic, RM = root methanolic, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

RD cell line when subjected to roots aqueous extract and glycoside FI. Table 8 demonstrated that stems and leaves methanol extracts and proteoglycan were more toxic after 72 h than 24 and 48 h on growth of RD cells. However, stems and leaves aqueous extracts, root methanol had the same effect at 48 and 72 h on growth of these cells and all previous extracts were more effective than 24 h. Glycoside FI had CC 50% values of
Table 8. Mean ± SE for the effect of exposure time to (SM, SA, LM, RM, RA, LA, Glycoside (FI) and proteoglycan) extracts of *C. arvensis* on the proliferation of RD tumor cells *in vitro* (observation OD).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Time (h)</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem methanol</td>
<td>0.695±0.005</td>
<td>0.561±0.038</td>
<td>0.364±0.043</td>
<td>0.0967</td>
<td></td>
</tr>
<tr>
<td>Stem aqueous</td>
<td>0.652±0.004</td>
<td>0.463±0.047</td>
<td>0.372±0.041</td>
<td>0.1075</td>
<td></td>
</tr>
<tr>
<td>Proteoglycan</td>
<td>0.690±0.001</td>
<td>0.474±0.052</td>
<td>0.357±0.046</td>
<td>0.1161</td>
<td></td>
</tr>
<tr>
<td>Leaves methanol</td>
<td>0.680±0.004</td>
<td>0.491±0.058</td>
<td>0.460±0.057</td>
<td>0.1440</td>
<td></td>
</tr>
<tr>
<td>Root methanol</td>
<td>0.762±0.004</td>
<td>0.580±0.051</td>
<td>0.559±0.053</td>
<td>0.1203</td>
<td></td>
</tr>
<tr>
<td>Root aqueous</td>
<td>0.757±0.001</td>
<td>0.739±0.021</td>
<td>0.680±0.035</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Leaves aqueous</td>
<td>0.669±0.001</td>
<td>0.449±0.027</td>
<td>0.392±0.044</td>
<td>0.1002</td>
<td></td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.392±0.002</td>
<td>0.348±0.053</td>
<td>0.336±0.051</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

SE=standard error. SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

Table 9. Mean ± SE for MI of RD tumor cells after 72 h treatment with glycoside (FI) extract of *C. arvensis* *in vitro*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration mg/ml</th>
<th>MI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>10.34±0.41</td>
</tr>
<tr>
<td>Glycoside extract</td>
<td>0.353</td>
<td>1.93±0.13</td>
</tr>
<tr>
<td></td>
<td>0.177</td>
<td>3.44±0.29</td>
</tr>
<tr>
<td></td>
<td>0.088</td>
<td>6.71±0.68</td>
</tr>
</tbody>
</table>

L.S.D (0.05) 1.409

SE = Standard error, LSD = least significant difference.

1.775, 0.870 and 0.706 mg/ml after exposure to 24, 48, and 72 h, respectively. The result of cytogenetic analysis showed a decrease in MI of RD tumor cell line treated with concentrations of 0.353, 0.177 and 0.088 mg/ml of glycoside FI extract as compared with control groups 1.93 ± 0.13, 3.44 ± 0.29, 6.71 ± 0.68 and 10.34 ± 0.41, respectively (Table 9).

**DISCUSSION**

**Cytotoxic effect of (aqueous and methanol) crude extracts, PGM and glycoside FI of *C. arvensis* on RD tumor cell line *in vitro***

The cytotoxic effect of all extracts of *C. arvensis* on RD tumor cell line varied depending on the extract and its concentration. The results showed that glycoside extract FI was more effective against the proliferation of RD tumor cell line. This result was supported by Mojab et al. (2003) where it was shown that glycoside components play an important role in cytotoxicity against cancer. The high inhibition activity of glycoside FI extract against the RD cell line may be explained by different mechanisms. Glycoside extracts had different inhibitory properties on potassium fluxes that clearly inhibit the potassium intake of those cells by inhibition of the Na-KATPase enzyme activity, which in turn leads to significant change in the permeability of the plasma membrane that allow the entry of compounds to the cell and disrupt the nitrogen base sequence of DNA (Chakravarty, 1976). Choi et al. (1994) found the anti-mutagenic capacity of glycoside dependent on free hydroxyl groups. Merfort et al. (1994) concluded that most of glycosides penetrate into human skin, making it a candidate for prevention and treatment of skin cancer.

Koishi et al. (1992) showed that quercitin inhibited production of heat shock proteins in several malignant cell lines, including colon cancer. Heat shock proteins form a complex with mutant p53, which allows tumor cells to bypass normal mechanisms of cell cycle arrest (Ranelletti et al., 1999). The obtained results of glycoside cytotoxicity may be attributed to the inhibition in the production of heat shock proteins. The ability of PGM extract of *C. arvensis* to reduce the proliferation of RD cells was in time-dependent manner, since its activity increased
after 48 and 72 h, and its effect was started at concentrations of 0.156 µg/ml, up to 10 mg/ml. Calvino (2002) prepared a novel proteoglycan mixture (PGM) extract from C. arvensis and their results showed that these extracts inhibited tumor growth and angiogenesis in chick embryo and improved lymphocyte. Toxicity of C. arvensis may be related to the presence of several types of alkaloids (pseudotropin) or other components of flavonoids, saponins, carbohydrate (AL-Edani, 1998). Winter (2008) showed that alkaloids reduced the proliferation of mouse lymphoblast cell line. Both leaves and stems methanol extracts showed high activity against the proliferation of RD tumor cell line at concentration of 0.156 mg/ml and up to 10 mg/ml, especially at 72 h. The current results were similar to that obtained by Sadeghi-aliaabadi et al. (2008) where they showed that chloroform, methanol and ethanol extracts of aerial parts of C. arvensis possessed high cytotoxic activity on Hela tumor cell line.

Awad et al. (2004) demonstrated that the roots contain low amounts of crude protein (18.7%), aspartic acid and alanine less than green parts and had high amounts of phenylalanine, which may explain the low activity of roots aqueous extract against the proliferation of RD cell line. Some root compounds have low ability to be absorbed by cell membranes (Marja, 2004). This low activity of root extract may be due to the resistance of the cell lines to its compounds. Lee et al. (2003) found that the tumor cells vary in their response to different drugs or crude extracts according to the types of cell membrane receptors. Our results revealed that the methanol roots extract had more toxicity than roots aqueous extract since the methanol had high polarity which could dissolve both polar and non-polar components which then can be actively passed through the plasma membrane. Cannell (1998) and Rajendran and Ramakrishnan (2009) found that the high polarity of methanol extract of Artocarpus heterophyllus was responsible for inhibiting nearly 100% of Hep2 cells. The other explanation for the differences in cytotoxic activity between aqueous and methanol extracts may be due to the presence of the calystegine alkaloid extracted by methanol (Russel, 1993). A study of Khanavi et al. (2009) supported the obtained results where they found that methanol extract from Stachys species contains the most potent antioxidant and had more activity than the aqueous extract.

Cytogenetic effect of glycoside (FI) extract of C. arvensis on the RD tumor cell line in vitro

The present experiment focused on determining the effect of glycoside FI extract on MI of RD cell line. Rhabdomyosarcoma tumor cell line showed a decrease in MI when treated with all concentrations (0.353, 0.177 and 0.088 mg/ml) of glycoside FI extract as compared with untreated cells (negative control group); their effects were concentration-dependent manner. The ability of glycoside FI to reduce the MI of RD tumor cell line might be explained by its contents of chemical constituents that had ability to reduce cell cycle progression. Amorim et al. (2000) found that the decrease in MI reflects the inhibition of cell-cycle progression and/or the loss of proliferative capacity. Cell cycle arrest in S-phase was found in human lung squamous carcinoma cells treated with glycoside extract (Leung et al., 2005). Wang et al. (2007) found that the glycoside isolated from two Asian plants Epimedium koreanum and Terminalia arjuna were traditionally used as anticancer medicines; they inhibit proliferation of MCF-7 (breast cancer) and HepG2 (liver cancer) cells in a dose-dependent manner.

Conclusion

Fraction I of glycoside caused high inhibition activity on growth of RD cell line after 24, 48 and 72 h. The aqueous roots extract had less inhibiting activity against the growth of RD cells. The Fraction I of glycoside showed high antimitotic effect on RD cell line with concentrations of 0.353, 0.177 and 0.088 mg/ml.

ACKNOWLEDGMENT

We express our deep appreciation and sincere thanks to the Head and Staff of the Iraqi Center for Cancer and Medical Genetic Research, Baghdad/Iraq. We wish to thank Assist. Prof. Dr. Iqbal J.A.-Assadi, Department of Chemistry, College of Science for her help in interpreting the IR and other biochemical advice.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


WHO (2002). Traditional medicine strategy launched. 80:610.

A study to document medicinal plants parts and practices in relation to sustainable use was performed in communities around the Miombo woodland of Urumwa in Tanzania. Data collection was based on semi-structured interviews and discussion with key stakeholders. G-tests were used to ascertain differences in medicinal plants practices between women and men. Roots were the commonly used parts for medicine followed by barks. In practice, boiling and grinding were the preferred methods for preparing remedies. Remedies are administered orally in decoctions form with lack of standardized dosages. Medicinal plants are collected from the reserve using both root digging and bark stripping methods. Local communities need sensitization through trainings on various aspects of medicinal plants practices especially issues of sustainable harvesting methods, simple processing, domestication and the importance of standardized dosages so as to improve work performance, ensure resource sustainability and contribute to development of the primary health care system in Tanzania.

**Key words:** Medicinal plants, practices, sustainability, Miombo woodlands, Tanzania.

**INTRODUCTION**

Traditional medical practices are important parts of the primary healthcare system in the developing world (Sheldon et al., 1997). Plant-based traditional knowledge has been recognized to be a tool in search for new sources of pharmaceuticals. Medicinal plants are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries. Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future (Pei, 2001). In Tanzania, traditional treatments using medicinal plants enjoy considerable popularity and are practiced by numerous healers all over the country, despite Western medicine being the mainstream of the health care system (Ruffo, 1990; Ishengoma and Gillah, 2002). By the late 1990's, Tanzania had more than 30,000 traditional healers who
who mainly operated in rural communities (Uri et al., 1996). Considering the population of Tanzania, Ishengoma and Gillah (2002) noted that at least one healer serves 750 people, whereas 50,000 people are served by one qualified medical doctor.

Though most practices and treatments in herbal medicine require specialists or professionals which are referred generally to as herbalists, self-care using plants is common in Tanzania. The plant parts used, preparation and administration of herbal medicines vary from one place to another. However, the knowledge of herbal medicines is gradually perishing, although some of the traditional herbal men are still practicing the art of herbal healing effectively.

Medicinal plant species throughout the tropics according to Akerele et al. (1991), Balick and Cox (1996), Lange and Schipmann (1997) and Leanman et al. (1999) are threatened in the wild due to over-exploitation. Unsustainable harvesting and land-use practices for Miombo land and products generally are now recognized as serious problems (Bodeker, 2002). The main threat (Maundu et al. 2004) is unsustainable harvesting practices, particularly ring-barking and uprooting. A well known example is the commercial exploitation of Warburgia salutaris (Bertol.f.) Chiov. bark from the miombo woodland of Southern Africa (Botha et al., 2004), which is commercially traded in the markets. The species is among the International Union for Conservation of Nature (IUCN) (2001) Red List species categorized as vulnerable and endangered in different countries in southern Africa.

Within rural communities, the main underlying cause of over-exploitation of medicinal plants is the combination of poverty and high unemployment due to falling per capita income in most African countries. Harvesting and the provision of medicinal plants to meet the urban demand have become an environmentally destructive activity (Williams et al., 2000), due to the development of a substantial network of rural commercial gatherers, herb traders, traditional healers and consumers. In response, non-professionals have turned to herbal harvesting and trading (and even treatment activity).

Like many other rural communities, the people around the Miombo woodlands of Tabora use medicinal plants for their primary health care. Ethnobotanically, these people have recently been explored (Augustino et al., 2011); however, there is no comprehensive account of the plants in terms of parts used and practices mainly sustainable harvesting, processing and preparations as well as dosages. In this paper we provide understanding of the medicinal plants practices in relation to sustainable use by communities around the miombo woodlands of Urumwa and Tanzania. In the context of this paper, medicinal plants practices are referred to as all activities included in the use of medicinal plants from preparations, processing up to the mode of administering the remedies in relation to sustainable use of medicinal plant resources. The findings are crucial in designing strategies to promote primary health care and ensure sustainable utilization of wild plant resources in the Miombo woodlands of Tanzania.

**METHODOLOGY**

**The study area**

The study was conducted between April to November, 2004 in six villages (that is, Igombaniolo, Isukamahela, Kasisi ‘A’, Masimba, Makuja mashariki and Ujerumani) around the Miombo woodland of the Urumwa Forest Reserve in Tabora-Uuyi District, Tabora Region (4 to 7° S, 31 to 34° E). The six villages were selected based on their closeness to the reserve and involvement in the Joint Forest Management (JFM) programme. Tabora region forms part of the vast central plateau of the mid-western part of Tanzania (Figure 1a), an area of generally low relief most of which lies between 1,100 and 1,300 m elevation (Acres et al., 1984), where about 61% of the vegetation covers of Tabora region is dry Zambezian Miombo woodland (White, 1983). The choice of the study area was based on the richness of its Miombo woodlands. The reserve and its surrounding villages (5° 08’ to 5° 14’ S, 32° 44’ to 32° 50’ E) are about 15 km south of Tabora municipality (Figure 1b) and cover an area of about 13,000 ha. The reserve is bordered by 12 villages collectively with an estimated population of about 22,500 (Mbawambo, 2000). A large proportion (approximately 80%) of Tabora’s urban population relies on the reserve for medicinal products.

Communities residing around Urumwa belong to two big ethnic groups that are Nyamwezi and Sukuma, though a small proportion of mixed tribes do exist. The main livelihoods of the locals in the study area are subsistence farming and livestock keeping. However, pitting, charcoal production and bee keeping are undertaken regularly (Mbawambo, 2000) and various non-timber forest products such as wild fruits and medicinal plants are collected and sold.

Semi-structured face-to-face interviews using a mixture of open and closed-ended questionnaires were conducted with herbalists (that is, traditional healers, medicinal plant sellers and traditional birth attendants), medicinal plant collectors and knowledgeable households in six villages close to Urumwa Forest Reserve (Table 1), to collect information on local plant names, uses, parts used and modes of preparation and administration. Voucher specimens were also collected, pressed, dried, botanically identified and deposited at Tabora Miombo Woodland Centre and the Institute of Traditional medicine Herbarium. Furthermore, several informal discussions with respondents and participant observation techniques were also employed to confirm the survey data and to gather additional information. Approach and entry to villages for data collection was through the village leadership, generally the Chairmen and Executive Secretaries, ensuring smooth running of day to day activities within the study area. Most respondents were generous in sharing their knowledge during the survey; however a few traditional healers refused to disclose their knowledge because they believe that once disclosed it will lose its effectiveness and also reflected the idea of a trade secret in traditional medicine system. Those who refused were not included in the interview and someone else was consulted.

A stratified sampling strategy based on gender in households of the six selected villages was used to select informants that is,
herbalists, medicinal plant collectors and household heads. In total, 115 informants (62 male and 53 female) were involved in the survey to explore local knowledge on use of medicinal plants; out of which 60 were herbalists, 6 medicinal plants collectors and 49 household heads. Based on the nature of the data in forms of responses in frequencies, G-tests of association were carried out to seek differences in medicinal plants practices knowledge between women and men around Urumwa. Assuming that men and women would have similar knowledge on practices of medicinal plants, the likelihood ratio statistic \( G \) was calculated as:

\[
G = 2 \times \sum \left( \frac{\text{observed frequency} \times \ln \left( \frac{\text{observed frequency}}{\text{expected frequency}} \right)}{\text{expected frequency}} \right)
\]

Calculated values were corrected for continuity by applying the William's correction factor (Sokal and Rohlf, 1995). \( P \) values were calculated using the common method as recommended by Bailey (1995) as follows:

\[
P = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}
\]

Where: \( (a+b), (c+d), \ldots, (b+d) = \text{Marginal values from 2 } \times \text{ 2 contingency table} \)

\( a+b+c+d = n = \text{Total sample size} \)

\( x! = 1, 2, 3, \ldots, (x-1) = \text{Factorial and } 0! = 1 \)

RESULTS

Plant parts used

The ethnobotanical information has been documented by Augustino et al. (2011), where 110 medicinal plant species (72% trees, 20% shrubs and 8% herbs) belonging to 37 families in 20 phylogenetic orders were recorded in the Miombo woodlands of Urumwa forest reserve. Out of the reported species, 99 were from inside and 11 outside the forest reserve. Roots have been observed to be the most plant parts commonly used for medicine by communities around Urumwa Forest Reserve. Bark and leaves were also used, while whole plants, fruits, seeds, twigs and exudates were rarely mentioned to be used (Table 2).

Processing and mode of preparation

For most of plant medicines used by Urumwa communities (that is, 72% trees, 20% shrubs and 8% herbs), processing was noted to begin with sun drying, followed sometimes with pounding after which the material is ground using a local grinding stone. For both males and females around Urumwa, boiling combined with grinding (Table 3) was the preferred method for preparing remedies; followed by boiling only (32%) and grinding only (10%). Nevertheless, G-tests of independence showed no gender difference at \( p = 0.05 \) on the preferred methods of preparing medicinal plant remedies at Urumwa.

Modes of administration

The majority of male and female respondents at Urumwa indicated to administer plant medicines in the form of decoctions (Table 4) with a remedy in powder form were reported only once. Oral administration through drinking,
Table 1. Sampling scale used during ethnomedical survey in villages around Urumwa, Tanzania.

<table>
<thead>
<tr>
<th>Village name</th>
<th>Traditional healers</th>
<th>Traditional birth attendants</th>
<th>Vendors</th>
<th>Collectors</th>
<th>Household</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Me</td>
<td>Fe</td>
<td>Me</td>
<td>Fe</td>
<td>Me</td>
<td>Fe</td>
<td>Me</td>
</tr>
<tr>
<td>Masimba</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>7</td>
<td>8</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Igombanilo</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Mtakuja mashariki</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Isukamahela</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Ujerumani</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Kasisi ‘A’</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>7</td>
<td>-</td>
<td>28</td>
<td>8</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

Me, represents ‘Male’ and Fe, represents ‘Female’

Table 2. Medicinal plant parts used for medicine by communities at Urumwa, Tanzania.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Number of taxa (out of 111)</th>
<th>% of total taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>101</td>
<td>91</td>
</tr>
<tr>
<td>Bark</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Leaves</td>
<td>49</td>
<td>44</td>
</tr>
<tr>
<td>Fruits</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Seeds</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Whole plant</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Twigs and exudates</td>
<td>1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Table 3. Plant remedies preparation methods in communities at Urumwa, Tanzania.

<table>
<thead>
<tr>
<th>Respondent category</th>
<th>Boil</th>
<th>Pound</th>
<th>Grind</th>
<th>Boil and Grind</th>
<th>Grind and Pound</th>
<th>Boil and pound</th>
<th>Boil, Grind, Pound</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>22 (41)</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>22 (41)</td>
<td>1 (2)</td>
<td>0</td>
<td>3 (6)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>Male</td>
<td>15 (24)</td>
<td>2 (3)</td>
<td>10 (16)</td>
<td>31 (50)</td>
<td>0</td>
<td>0</td>
<td>4 (7)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Totals</td>
<td>37 (32)</td>
<td>5 (4)</td>
<td>12 (10)</td>
<td>53 (46)</td>
<td>1 (1)</td>
<td>0</td>
<td>7 (6)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages of row total.
Table 4. Forms of remedies used by communities around Urumwa, Tanzania.

<table>
<thead>
<tr>
<th>Respondent category</th>
<th>Response category</th>
<th>Decoctions</th>
<th>Infusions/ concentrates</th>
<th>Powders</th>
<th>Others*</th>
<th>Mixtures</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td>35 (66)</td>
<td>4 (7)</td>
<td>1 (2)</td>
<td>10 (19)</td>
<td>3 (6)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>36 (58)</td>
<td>6 (10)</td>
<td>0</td>
<td>16 (26)</td>
<td>4 (6)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>71 (62)</td>
<td>10 (9)</td>
<td>1 (4)</td>
<td>26 (23)</td>
<td>7 (6)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages of rows total, * mostly poultices and protective charms.

Table 5. Modes of administering remedies used around Urumwa, Tanzania.

<table>
<thead>
<tr>
<th>Respondent category</th>
<th>Response category</th>
<th>Oral</th>
<th>External</th>
<th>Oral and nasal</th>
<th>Oral and anal</th>
<th>Oral and external</th>
<th>All modes</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td>23 (43)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>8 (15)</td>
<td>13 (25)</td>
<td>6 (11)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>32 (52)</td>
<td>0</td>
<td>2 (3)</td>
<td>3 (5)</td>
<td>19 (31)</td>
<td>6 (9)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>55 (48)</td>
<td>1 (2)</td>
<td>4 (3)</td>
<td>11 (9)</td>
<td>32 (28)</td>
<td>10 (12)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are frequencies of row total.

Table 6. Dosage knowledge around Urumwa communities, Tanzania.

<table>
<thead>
<tr>
<th>Respondent category</th>
<th>Response category</th>
<th>Standardized</th>
<th>Informal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td>20 (38)</td>
<td>33 (62)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>30 (48)</td>
<td>32 (52)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>50 (43)</td>
<td>65 (57)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.

chewing and or mouthwash (Table 5) was typical (48%) for both female and male at Urumwa, followed by mixture of oral and external modes as mainly massage, baths, steaming and smoking.

Plant remedies dosages

Neither female nor male respondents consistently used standardized dosages when administering plant remedies to patients (Table 6). Dosage for most local communities is generally determined subjectively and depends on the severity of the symptoms. Where described as ‘standardized’, administration of remedies was done as a specific quantity for a specified period of time and the patient had to continue with the dose even if he/she felt better soon after taking the medicine. For example, ½ cup of tea × 3 × 5 days was commonly mentioned for treating diseases like intestinal worms and venereal diseases.

In the case of 'informal' dosages, however, no quantity specification was provided, the patient had to estimate a small quantity to take and was expected to stop using the remedy soon after he/she feels recovered from the ailment concerned.

Medicinal plants harvesting

Most medicinal plants used by communities around Urumwa grow wild and were collected mainly from the forest reserve with few cultivated around homesteads. Root digging and bark stripping in combination was the most reported method used for harvesting medicinal plant parts in communities around Urumwa. As a single procedure, root digging was reported most frequently (Table 7).

DISCUSSION

For the case of Urumwa surrounding communities, the
medicinal plants practices seem to be dominated by the use of roots to prepare decoctions for oral administration. However, lack of any standardized dosage noted among users in both men and women is striking. On one hand, it implies not only confidence in the informal limits within which traditional medicines are administered but also suggests inadequate attention to potential risks in terms of safety and quality of plant medicines. A measure of complacency may probably be resulting from the rather high level of illiteracy.

Earlier studies in Tanzania have also noted the lack of standardized dosages for treatment (Maximillian et al., 2001; Kitula, 2001, 2007) administered by most herbalists and by other household members who all tend to rely on long term experience that probably does impose some regulation. Yineger et al. (2008) noted lack of precision in measurements for most traditional healers when administering plant remedies. Certainly, Giday et al. (2003) noted in Zay communities of Ethiopia that dosages took account of age and the physical and health condition of the patient. Similar observations on dosages are noted by Abebe and Ayehu (1993) also in Ethiopia, in addition to of socio-cultural explanation of the illness, diagnosis and experience of individual herbalist in prior to giving remedies to the patient. Nevertheless, potential side effects are sometimes not considered probably due to lack of knowledge. According to Hillebrand (2006) and Kitula (2007), the lack of consistent dosage may be potentially dangerous as some of the species could have a high degree of toxicity, over dose might cause serious health problems for patients.

Sun drying as a processing method seems to be done un-hygenically on bare ground or using plastic mesh materials at Urumwa. This makes the product potentially harmful as dust may contaminate it, while fungi and bacteria might grow on the plant tissue. The process also seems to be wasteful as much plant materials are lost during all stages of processing. The need to introduce simple processing technology to reduce post-processing loss and conserve the plant material is highly recommended to communities.

Boiling combined with grinding is the preferred method for preparing remedies at Urumwa. Results seem to agree with Kitula (2007), Medius (1998) and Marshall (1998) in traditional medicine that boiling is believed to be efficient in extracting active ingredient and for hygienic reasons. Similarly in Ghana, a study by Asase et al. (2005) observed that the majority of the herbal preparations involved boiling the plant material and then drinking the extract; with none of the people interviewed providing any information about how the treatment might be standardized. According to Anfom (1986) and Sofowora (1982), lack of standardization and quality control is seen as one of the main disadvantages of traditional medicine.

The roots, leaves and barks of many Miombo species have been reported to be used in health care, both as medicine and for magic (Gelfand et al., 1985). However, the dominant use of roots at Urumwa apparently reflects belief that roots have higher concentrations of remedial elements, a belief also reported by Kitula (2007) around Udzungwa Mountains and Makonda et al. (2000) in Kilosa, Bagamoyo and Geita Districts of Tanzania. The clear dominance of roots is not always the case in traditional medicine. For instance, a study by Hamisy et al. (2000) around Uluguru Mountains observed wide use of leaves as well as roots. Leaves were actually the dominant plant parts used in areas studied in Ethiopia (Giday et al., 2003) and Uganda (Tabuti et al., 2003). It has been postulated (Dhillion and Amundsen, 2000; Dhillion and Gustad, 2003; Shrestha and Dhillion, 2003) that the selection of perennial plant parts such as roots, tubers, bark and stem or reproductive plant parts, especially of woody or slow growing species, for use as herbal medicines can threaten plant populations or species viability. This assumption is supported by respondents’ observations, as some species, such as Ekebergia benguelensis DC. and Cassia abbreviata Oliv. were reported to become rare (that is, difficult to find in the forest reserve as their population is perceived to decline based on the frequency of report from interview) inside Urumwa Forest Reserve due to unsustainable harvesting intensities and practices of mainly whole plant removal. However, the effect of harvesting upon the medicinal plant populations has not been assessed in Urumwa, necessitating the need to do so prior to the development of cultivation/domestication plans.

Table 7. Harvesting methods used in communities around Urumwa Forest Reserve, Tanzania.

<table>
<thead>
<tr>
<th>Respondent category</th>
<th>Root digging</th>
<th>Bark stripping</th>
<th>Root digging and leaves collection</th>
<th>Root digging and Bark stripping</th>
<th>All methods</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>18 (34)</td>
<td>1 (2)</td>
<td>5 (9)</td>
<td>32 (42)</td>
<td>9 (13)</td>
<td>53 (100)</td>
</tr>
<tr>
<td>Male</td>
<td>19 (31)</td>
<td>1 (2)</td>
<td>2 (3)</td>
<td>28 (45)</td>
<td>12 (19)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Totals</td>
<td>37 (32)</td>
<td>1 (2)</td>
<td>7 (6)</td>
<td>50 (43)</td>
<td>19 (16)</td>
<td>115 (100)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are percentages.
The future availability of medicinal plants in the Miombo woodlands of Urumwa might seriously be threatened in future if root digging and debarking will be entertained for commercial interest by users. According to Giday et al. (2003), collection of leaves does not pose a great danger to the existence of an individual plant when compared with the collection of underground part, stem, bark or whole part. Nevertheless, Abebe and Ayehu (1993) argued that the popularity of roots including bulbs and rhizomes, barks and stems has serious consequences from both ecological point of view (that is, affecting plant viability) as well as the survival of the medicinal species in the wild. It is suggested that the government should pay attention to plant species which utilize roots and barks for medicinal purposes including studies on specific management in order to ensure the resources’ sustainability interms of productivity and yield in the Miombo of Urumwa.

CONCLUSION AND RECOMMENDATIONS

The traditional medicinal practice by men and women at Urumwa is dominated by the use of roots and lack of standardized dosage for most of plant remedies administered. These are sensitive matters which could hinder the development of traditional medicine system unless serious actions are taken. Sustainable harvesting practices in addition to domestication need to be emphasized and recognized by communities as the most important conservation strategy, given their current and potential contributions to the primary health care services, local economies and their greater value to harvesters over the long term. Local communities also need to be sensitized through provision of trainings on issues of simple processing technology and the importance of having standardized dosages to improve their work performance and contribute to the development of primary health care services. A quantitative study to assess the status of potential medicinal plants at Urumwa is vital before designing conservation strategies.

ACKNOWLEDGEMENTS

We are grateful to the communities around Urumwa Forest Reserve in Tabora Region, Tanzania who generously shared their traditional knowledge with us. We are also indebted to the Commonwealth Scholarships Commission for funding the senior author’s research activities.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


