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Phytochemical Compounds and Antimicrobial Activity of Extracts of Aspilia Plant (*Aspilia mossambicensis)* (Oliv) Wild

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Abstract: Phytochemical compounds and anti-microbial properties of the methanol extracts of *Aspilia mossambicensis* (Compositae) were studied between March and April, 2007 at Maseno University, Kenya. The bacterial used for the antimicrobial analysis consisted of clinical strains of *Streptococcus pyogenes* (gram positive) and *Salmonella typhi* (Gram negative) bacteria and one strain of fungi (*Aspergillus niger*). The methanol extract was active against the three microorganisms. Leaves extracts showed greater microbial growth inhibition in comparison to root extracts. The phytochemical screening for the plant leaves and roots of *A. mossambicensis* revealed the presence of active compounds of flavonoids, alkaloids, saponin, steroids and anthraquinones. The results from the present study have shown that species of *Aspilia mossambicensis* have considerable activity against the gram negative bacterium *Salmonella typhi*, gram positive bacterium *Streptococcus pyogenes* and one fungal strain *Aspergillus niger*. *Streptococcus pyogenes* experienced lesser growth inhibition with root extracts compared to the other two microbes. It is possible that the growth inhibition observed in the study occurred due to presence of different chemical compounds. Absence of aldehydes in roots but not in leaves could explain the greater growth inhibition of *S. typhi* and *A. niger*. Pit method was a better method for testing antimicrobial activity than disc method in this study.

Key words: Antimicrobial activity, *Aspilia mossambicensis*, phytochemical compounds, Kenya

INTRODUCTION

Plants naturally synthesize several carbon compounds, basically for physiologic functions or for use as chemical weapons against disease organisms, insects and predators (Fatope, 1995). The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with an aim of discovering new clinically useful and commercially important plant products (Dewick, 1997). It is estimated that 70-80% of all over the world largely depend on traditional herbal medicine to meet their primary health care needs (Hamayun *et al*., 2006). The global demand for herbal medicine is growing (Omino and Kokwaro, 1991; Muregi *et al*., 2003; Zowai *et al*., 2003). While plant species used in traditional medicines continue to be reliable sources for discovery of useful compounds, screening of plants growing under various environmental conditions could provide another source for compounds with antimicrobial activities (Njoroge and Newton, 1994; Rajakaruna *et al*., 2002; Muregi *et al*., 2003; Zowai *et al*., 2003; Adebooye and Opabode, 2004; Duraipandiyan *et al*., 2006). Biological and pharmacological activities of phytochemical compounds take into account different parameters and factors such as species, ecological factors and environmental conditions. Thus, each plant species will present a profile which it will express differently among these factors. Phenological age of the plant, percent humidity of the harvested material and method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Rajakaruna *et al*., 2002). There is a wide variation in the susceptibility of organisms to toxic compounds. It is probable that a large number of plants with biological activities remain untested.

Aspilia plant (*Aspilia mossambicensis*) is widely spread in south, south west and west of Kenya from coast to Lake Victoria. Due to diversity of ecological conditions, the chemical composition of the plants is known to vary and due to this reason plants have been used to treat different diseases in different places (Njoroge and Newton, 1994; Agnew and Shirley, 1994; Masinde, 1996).

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The plant belongs to the family of Compositae (Asteraceae) and genus *Aspilia*. There are reports that the plant possess antimalarial activity against *Plasmodium falciparium*, galactogogue activity and is used to alleviate menstrual cramps (Offulla *et al*., 1996). Literature search indicate that this plant has been used traditionally by many African communities to treat several diseases (Kokwaro, 1976; Johns *et al*., 1990), such as cystitis and gonorrhea, abdominal pains, backache and the root decoction is normally given to breast feeding mothers to increase milk production. Pounded leaves are rubbed on the skin and freshly cut wounds for faster healing of wounds and ringworms. Pounded leaves decoction is drunk in order to treat intestinal worms including hookworms (Kokwaro, 1976; Omino and Kokwaro, 1991). Among the Kamba community of Kenya, pounded leaves decoction is applied to circumcised young men for faster healing of the wounds (Musyimi, personal communication). Some phytochemical constituents of this plant have been reported to have medicinal or antimalarial properties (Kokwaro, 1976; Offulla *et al*., 1996). However there are no reports of antimicrobial activity of this plant in the literature. This study aimed at isolating, identifying and evaluating the antimicrobial properties of the compounds from *Aspilia mossambicensis* (Oliv) wild species known to treat diseases in rural communities in Kenya.

MATERIALS AND METHODS

Field collection and extraction: Plant roots and the leaves were collected from areas surrounding Maseno University, Kenya between March and April 2007. The materials were cleaned off adhered soil or dust in the field by shaking and were placed inside polythene paper bags and taken to the Maseno University laboratory where the tissue specimens were washed with distilled water and allowed to dry under shade. The voucher specimens in duplicate were deposited in the herbarium of Maseno University. Leaves and roots, of *Aspilia mossambicensis* (oliv*)* wild species were air dried at room temperature for 2 weeks. The roots and leaves were subsequently ground into fine powder using an electric blender yielding 300 and 500 g, respectively and cold extracted with methanol then filtered under vacuum using a 0.2 mm Nalgene filter unit. The resulting extracts were concentrated to remove the methanol using the rotary vapour at 40°C. The roots and leaves extracts were dried and subsequently weighed to yield a crude extract of 31.8 and 19.7 g, respectively. Extracts were stored in refrigerator until needed for analysis.

Phytochemical screening: The qualitative chemical analysis of the powders was carried out for the determination of the presence of anthraquinone, saponins, steroids and alkaloids, Aldehydes and ketones. Phytochemical screening was done according to Akinyemi *et al*. (2005) and Abulude (2007).

Isolation of pure compounds: The methanol extract (15 g) was chromatographed on silica gel column eluting with n-hexane: ethyl acetate (90:10) according to Kaberia *et al*. (1999). Most of the chemicals used were of analytical grade. Silica gel column (60 g, si 60, 40-63 μm) was from Merck, Germany and commercially prepared TLC plate (GmbH and Co. D-3440) from Eschwege, Germany. Seven, 25 mL fractions were collected from the leaves and 9 fractions were collected from the roots. The R_f values of the bioactive fraction in the sample were determined.

Test microorganisms: The clinical isolates were obtained from biomedical school of Maseno University, Kenya. Test microorganisms consisted of one gram positive bacteria (*Streptococcus pyogenes*) and one gram negative bacteria (*Salmonella typhi*) and one fungal strain (*Aspergillus niger*).

Preparation of the culture media: Nutrient agar pH 7.4 and Sabouraud dextrose agar pH 5.4, all products of oxoid laboratories, England were used in this study. Thirty nine grams of Sabouraud dextrose agar and 28 g of nutrient agar were dissolved in 1 L of water and heated to dissolve the contents completely. The nutrient agar and Sabouraud dextrose agar petri dishes were prepared by pouring 15 mL of molten media into sterile petri dishes and allowed to solidify for 5 min. The media, distilled water and petri dishes were sterilized separately by autoclaving at 120°C at 1 bar pressure for 20 min.

Determination of antimicrobial activity: Extracts from the two plant parts (leaves and roots) were diluted in hexane (2 g of the dried filtrate was reconstituted with 10 mL of 100% hexane to prepare stock solution). Different concentrations of the plant extracts were prepared by diluting the stock extract to hexane according to the following ratios 1:9 (10%), 1:18 (5%) and 1:32 (2.5%). The control had n-hexane alone without any extract to nullify the effect of the solvent on the test organisms. Two methods were used; this is pit and disc methods according to Murthy and Nagodra (1977) and Rajakaruna *et al*. (2002). Susceptibility testing was carried out by measuring the inhibitory zone diameters on the Nutrient Agar (NA) and Sabouraud dextrose agar using

conventional paper disc method and pit method. The inhibitory zone distances and rounded off to the nearest whole numbers (mm) for analysis.

Pit method: A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar media, into which 0.1 mL of the prepared plant extracts of various concentrations were added. The test microorganisms were introduced on the media around the pit using applicator sticks bearing cotton wool at the tips. Three replicates for each microbe and plant extract concentration were used. The petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The clear zone of growth inhibition formed around each pit was measured to the nearest millimeter using a transparent ruler.

Disc method: Circular paper discs 8 mm diameter were cut out from Whatman No. 1 filter paper using a paper punch and each dipped in a known concentration of the plant extracts for about 2 min, then were gently transferred to the centre of the inoculated agar media. Petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The diameters of growth inhibition zones were measured using a ruler and compared to the n-hexane control disc to nullify the effect of the solvent on the growth of the test organisms. All work was carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 2000).

Data analysis: Data collected were subjected to analysis of variance (ANOVA) using SAS statistical package. Means were separated and compared at ($p<0.05$).

RESULTS

Phytochemical screening of the plant parts (Table 1) showed that the plant contains flavonoids, alkaloids, steroids and anthraquinones, ketones and aldehydes. Root extracts lacked aldehydes unlike the leaves which had both ketones and aldehydes. Flavonoids were only present in roots extracts. There were a lot of sterols in the roots extract than in the leaves extract. Higher concentrations of alkaloids were present in both root and leaves extract. *Meta* and *Ortho* hydroxyls groups were present in both roots and leaves extracts while *Para* hydroxyl groups were only present in leaves, this confirmed the presence of carbohydrates in the plant extracts. Elution of the column with n-hexane and ethyl acetate led to isolation of fifteen fractions of R_f values of uncharacterized active compounds (Table 2). Seven active compounds were isolated from the leaves and nine active compounds from the roots. There were significant ($p<0.05$) differences between the plant parts, extracts concentration, test microorganisms and bioassay methods used. Pit method had significantly greater growth inhibition (Table 3) compared to disc method. Both plant extracts were found to possess antimicrobial activities. The leaves extracts had higher growth inhibitory activity compared to root extracts (Table 4). The zones of growth diameter inhibition obtained with respect to the test organisms were higher for *A. typhi*, followed by *A. niger* and *S. pyogenes*, respectively.

Table 1: Phytochemical screening of secondary metabolites present in the plant parts extracts of *Aspilia mossambincensis*

Phytochemical groupings	Leaves	Roots
Flavonoids	-	++++
Anthraquinone glycosides	++	+++
Steroid glycosides	++	++++
Alkaloids	++++	++++
Safonin glycosides	++	++
Carbonyl compounds		
Ketone	+	+
Aldehyde	+	-

-: Absent, +: Present, ++: Low concentration, +++: Moderate concentration, ++++: High concentration

Table 2: The R_f values of active compounds isolated from the methanol plant extracts of *Aspilia mossambincensis* using thin layer chromatography

Plant part extract	Spot No.	Distance of solvent from origin (cm)	Distance of spot from origin (cm)	R_f value
Leaves	1	6.0	2.0	0.30
	2	6.0	1.5	0.25
	3	6.1	2.6	0.42
	4	6.0	3.5	0.58
	5	5.8	3.4	0.59
	6	6.1	2.9	0.48
	7	6.0	4.5	0.75
Roots	1	5.8	1.5	0.26
	2	5.8	2.3	0.40
	3	5.7	3.0	0.52
	4	5.7	2.1	0.37
	5	5.9	3.3	0.56
	6	6.0	1.8	0.30
	7	5.7	2.7	0.48
	8	6.1	1.5	0.25
	9	5.7	4.0	0.61

Table 3: The comparison of the inhibitory effects of the root extracts using the two methods of microbial tests on the test organisms

Microorganism	Pit method (mm)	Disc method (mm)
S. typhi	6.25±0.41	3.75±0.41
S. pyogenes	2.58±0.41	2.17±0.41
A. niger	5.83±0.41	5.25±0.41

Data values are means of three replicates±SE

Table 4: Analysis of mean data of the antimicrobial activity of *Aspilia mossambincensis* extracts, comparison of two methods of microbial tests and plant part used

Variables	Growth inhibition diameter (mm)
Microbe	
Salmonella typhi	5.0833a
Streptococcus pyogenes	4.6875ab
Aspergillus niger	4.2708b
LSD	0.5768
Extract concentration	
10%	9.6111a
5%	5.6389b
2.5%	3.3889c
Control	0.0833d
LSD	0.6661
Plant part	
Leaf	5.0556a
Root	4.3056b
LSD	0.4710
Method	
Pit method	6.0972a
Disc method	3.2639b
LSD	0.4710

Means followed by different letter(s) down the column are significantly different at $p<0.05$. Data values are means of three replicates

DISCUSSION

Medicinal plants have been the subject of human curiosity and need (Omino and Kokwaro, 1991; Masinde, 1996; Abulude, 2007; Khalil and Dababneth, 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolics compounds (Weimann and Heinrich, 1997; Atindehou *et al*., 2002; Oomah, 2003; Edeoga *et al*., 2005). The antimicrobial activity of the methanol extract appear to have a broad spectrum of activity, since both Gram positive and gram-negative bacterial and fungi were sensitive to the extracts. Many alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Shelton, 1991). The zone of inhibition measured for *Salmonella typhi*, a gram positive bacterium using pit method was greater compared to *S. pyogenes* a gram positive bacterium, indicating a greater degree of activity against the bacterium. Leaf extracts were more active than those from roots; probably because they had higher concentration of the active compounds than the roots or it may be due to absence of aldehydes in the roots as was evident from the study (Table 1). More interesting, the root extracts had higher fractions of active compounds as determined by thin layer chromatography (Table 2). The relatively greater growth inhibition caused by the leaf extract was as a result of the active constituents in leaves acting in a more synergistic manner. Interestingly, *S. pyogenes* experienced lesser inhibition with the root extracts compared to the other two microbes (Table 3 and 4). There is a possibility that growth inhibition of the microbes was due to different chemical compounds. Absence of aldehydes in roots but not in leaves extracts could explain the greater growth inhibition of *S. typhi* and *A. niger*. The toxic phytochemical compounds could either damage the DNA or inhibit the synthesis of proteins in these organisms (Fatope, 1995). *Artemisia capillaries*, a plant in the same family has been used in Chinese medicine to prevent or treat liver diseases (Lien and Li, 1983; Wang *et al*., 2007). Phytochemical compounds are very heterogeneous mixtures of single substances acting in a synergistic or antagonistic manner. Mixtures of active constituents show a broad spectrum of biological and pharmacological activity (Robinson, 1967; Coelho-de-Souza *et al*., 1998; Atindehou *et al*., 2002).

Antimicrobial properties exhibited by the plant could be due to presence of alkaloids, flavonoids and carbohydrates (*Meta* and *Para*-hydroxyl compounds) present in the plant extracts (Erdogrul, 2002; Mostahar *et al*., 2007). Many alkaloids have pharmacological effects. Alkaloids have been used to treat diseases like malaria, pain killers and managing heart diseases (Oomah, 2003). Alkaloids are derivatives of amino acids and include morphine, atropine, quinine, Cytochalasine, berberine, cocaine and nicotine. Flavonoids (rutin and anthocyanins), phenolics, polyphenolic compounds and saponins have biological and pharmacological activities on human health (Hartung, 1990). Fungitoxic phytochemical compounds include the phenolics compounds such as medicarpin, matteucinal, scopolin, chlorogenic acid and scopoletin (Fatope, 1995). Major flavonoid compounds such as rutin and its aglyconequercetin have beneficial biological activity, such as in antagonizing the increase of capillary fragility associated with hemorrhagic disease (Griffith *et al*., 1944; Abulude, 2007), reducing hypertension and anti-carcinogenic activity (Yang *et al*., 2000). Biological activities of many other flavonoids, polyphenols or phenolics compounds have been also reported (Edenharder and Grünhage, 2003; Wang *et al*., 2003). Flavonoids and hydroxystilbenes act by inhibiting enzymes that regulate cell proliferation (Fatope, 1995). The occurrence of hydroxyl groups in methanol extracts could enhance the likelihood of intramolecular reactions such as the formation of cyclic acetals and anhydroethers (Mann, 1994). It is possible that carbohydrates present in the extracts somehow facilitated growth of the microorganisms and hence antagonizing the antibacterial activity of the active compounds in the extracts.

The susceptibility of *S. typhi*, *S. pyogenes* and *A. niger* to the methanol extracts is an indication that the plant is suitable for use as a source of both antibacterial and antifungal compounds. The antimicrobial activities demonstrated by crude extracts of *A. mossambicensis* may, therefore explain some of the previous claims about this plant for the treatment of diseases like dysentery and respiratory tract infections (Kokwaro, 1976; Omino and Kokwaro, 1991). The antimicrobial properties exhibited by these plants could be traced to its possession of alkaloids and flavonoids noted for their numerous biological activities such as anti-inflammatory, vasoprotective and antithrombitic effects (Oomah, 2003; Mostahar *et al*., 2007). There are no reports of toxic effects of *A. mossambicensis* used in different African communities including Kenya for various ailments, hence phytochemical studies should be intensified to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity. Research is still in progress to find out the active compounds in the fractions of *A. mossambicensis*.

CONCLUSIONS

The present study with *A. mossambicensis* has confirmed antimicrobial activity against gram negative bacterium *S. typhi* and gram positive bacterium *S. pyogenes*, including the fungal pathogen *A. niger*. Leaves extracts showed greater growth inhibition than roots. *Streptococcus pyogenes* showed the lesser growth inhibition with root extracts in comparison with the other two organisms, suggesting that the growth inhibition was caused by different active compounds. Roots lacked aldehydes which were present in leaves extract; therefore aldehydes could be the compounds responsible for greater growth inhibition of *S. typhi* and *A. niger*. Antimicrobial activity of the plant could have been caused by saponins, flavonoids, anthraquinones, carbohydrates and alkaloids present in the plant extracts. The results suggest that the extracts of *A. mossambicensis* could be used as a source of cheaper substitute for conventional drugs. The results are guarantee of further verification using various organic and water extraction compounds, more sensitive and sophisticated bioassay techniques.

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REFERENCES

Abulude, F.O., 2007. Phytochemical screening and mineral contents of leaves of some Nigerian woody plants. Res. J. Phytochem., 1: 33-39.

Adebooye, O.C. and J.T. Opabode, 2004. Status of conservation of the indigenous leaf vegetables and fruits of Africa. Afr. J. Biotechnol., 3: 700-705.

Agnew, A.D.Q. and A. Shirley, 1994. Upland Kenya Wild Flowers. 2nd Edn., University of Walas, Aberystewyth, pp: 207-216.

Akinyemi, O.K., O. Oladapo, C.E. Okwara, C.C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement Altern. Med., 5: 6.

Atindehou, K.K., M. Koné, C. Tenneaux, D. Traore, K. Hostettman and M. Dosso, 2002. Evaluation of the antimicrobial potential of medicinal plants from the Ivory Coast. Phytother. Res., 16: 497-502.

Coelho-de-Souza, A.N., D.N. Criddle and J.H. Leal-Cardoso, 1998. Selective modulatory effects of the essential oil of *Croton zehntneri* on isolated smooth muscle preparations of the guinea pig. Phytother. Res., 12: 189-194.

Dewick, P.M., 1997. Medicinal Natural Products: A Biosynthetic Approach. John Wiley and Sons, Ltd., England, UK, pp: 1-466.

Duraipandiyan, V., M. Ayyanar and S. Ignacimuthu, 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern. Med., 6: 35 [http://www.biomedcentral.com/1472-6882/6/35].

Edenharder, R. and D. Grünhage, 2003. Free radical scavenging abilities of flavonoids as mechanism of protection against mutagenicity induced by tert-butyl hydroperoxide or cumene hydroperoxide in *Salmonella typhimurium* TA102. Mutat. Res., 540: 1-18.

Edeoga, H.O., D.E. Okwu and B.O. Mbaebie, 2005. Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol., 4: 685-688.

Erdogrul, O.T., 2002. Antibacterial activities of some plant extracts used in folk medicine. Pharm. Biol., 40: 269-273.

Fatope, M.O., 1995. Phytocompounds: Their bioassay and diversity. Discov. Innov., 7: 229-236.

Griffith, Jr., J.Q., J.F. Couch and M.A. Lindauer, 1944. Effect of rutin on increased capillary fragility in man. Proc. Soc. Exp. Biol. Med., 55: 228-229.

Hamayun, M., S.A. Khan, H. Kim, C.I. Na and I. Lee, 2006. Traditional knowledge and *ex situ* conservation of some threatened medicinal plants of Swat Kohistan, Pakistan. Int. J. Bot., 2: 205-209.

Hartung, A.C., M.G. Nair and A.R. Putnam, 1990. Isolation and characterization of phytotoxic compounds from asparagus (*Asparagus officinalis* L.) roots. J. Chem. Ecol., 16: 1707-1718.

Johns, T., J.O. Kokwaro and E.K. Kimanani, 1990. Herbal remedies of the Luo of Siaya District, Kenya: Establishing quantitative criteria for consensus. Econ. Bot., 44: 369-381.

Kaberia, F., C.P. Kiprono and M.D. Wambua, 1999. Bioactive extracts from *Senecio lyratus*. J. Agric. Sci. Technol., 2: 77-79.

Khalil, A.B. and B.F. Dababneth, 2007. Inhibition of phytopathogenic fungi by extracts from medicinal plants in Jordan. J. Biol. Sci., 7: 579-581.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, Nairobi, pp: 58-59.

Lien, E.J. and W.Y. Li, 1983. A survey of Chinese herbs V: *Artemisia capillaries* Thunb. (yinchen, Inchinko). Bull. Orient Healing Arts Inst. USA., 8: 18-25.

Mann, J., 1994. Carbohydrates. In: Natural Products: Their Chemistry and Biological Significance. Mann, J., R.S. Davidson, J.B. Hobbs, D.V. Banthorpe and J.B. Harborne (Eds.), Longman Group UK., Ltd., London, pp: 7-67.

Masinde, P.S., 1996. Medicinal plants of the Marachi people of Kenya. In: Proceedings of the XIVth AETFAT Congres Wageningen, The Netherlands; pp: 747-750.

Mostahar, S., P. Katun and A. Islam, 2007. Synthesis of two vanillin ring containing flavone by different methods and study of their antibacterial and antifungal activity. J. Biol. Sci., 73: 514-519.

Muregi, F.W., S.C. Chhabra, E.N.M. Njagi, C.C. Lang' at Thoruwa, W.M. Njue, A.S.S. Orago, S.A. Omar and I.O. Ndiege, 2003. *In vitro* antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potential. J. Ethnopharmacol., 84: 235-239.

Murthy, M.S. and M.T. Nagodra, 1977. Allelopathic effects of *Aristida adscensionis* on rhizobium. J. Applied Ecol., 14: 279-282.

Njoroge, G.N. and L.E. Newton, 1994. Edible and poisonous species of Cucurbitaceae in the Central Highlands of Kenya. J. E Afr. Nat. Hist., 83: 101-115.

Offulla, A.V., G.M. Rukunga, G.M.M. Chege, F. Kiarie, C.N. Muthaura, J.I. Githure and W.M. Kofi-Tsekpo, 1996. Antimalaria activity of fractions isolated from *Albizia gummifera* and *Aspilia mossambicensis* crude extract. Afr. J. Health Sci., 3: 44-46.

Omino, E.A. and J.O. Kokwaro, 1991. Ethnobotany of Apocynaceae species in Kenya. J. Ethnopharmcol., 40: 167-180.

Oomah, D.B., 2003. Isolation, characterization and assessment of secondary metabolites from plants for use in human health. PBI Bull. No. 1.

Rajakaruna, N., S.C. Harris and G.H.N. Towers, 2002. Antimicrobial activity of plants collected from Serpentine outcrops in Sri Lanka. Pharm. Biol., 40: 235-244.

Robinson, T., 1967. The Organic Constituents of Higher Plants, Their Chemistry and Interrelationships. Burgress Publishers Company, USA.

Shelton, R.M., 1991. *Aloe vera*. Its chemical and therapeutic properties. Int. J. Dermatol., 30: 679-683.

Wang, M., Y. Tadmor, Q.L. Wu, C.K. Chin, S.A. Garrison and J.E. Simon, 2003. Quantification of protodioscin and rutin in asparagus shoots by LC/MS and HPLC methods. J. Agric. Food Chem., 51: 6132-6136.

Wang, R., J. Kong, D. Wang, L.L. Lien and E.J. Lien, 2007. Review: A survey of Chinese herbal ingredients with liver protection activities. Chinese Med., 2: 5 [http://www.cmjournal.org/content/2/1/5].

Weimann, C. and M. Heinrich, 1997. Indigenous medical plants in Mexico: The example of the Nahua (Sierra de Zongolica). Pharm. Biol., 110: 62-72.

WHO (World Health Organisation), 2000. General guidelines for methodologies on research and evaluation on traditional medicine. WHO/EMD/ TRM/2001.1, pp: 1-70.

Yang, K., S.A. Lamprecht, Y. Liu, H. Shinozaki, K. Fan, D. Leung, H. Newmark, V.E. Steele, G.J. Kelloff and M. Lipkin, 2000. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis, 21: 1655-1660.

Zowai, J., F.M. Muli, H.I. Boga, S.C. Chhabra, W.M. Kofi, J.O. Oudo, E. Juma and W.A. Githui, 2003. The *in vitro* determination of the potency of selected Kenyan medicinal plants traditionally used to treat tuberculosis and other bacterial infections. In: Proceedings of the Second National Congress on Quality Improvement in Health Care, Medical Research and Traditional Medicine Nairobi, Kenya, Nov. 24-28.